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  (FILE 'HOME' ENTERED AT 20:56:10 ON 22 JUN 2003)

    FILE 'CAPLUS, USPATFULL' ENTERED AT 20:56:23 ON 22 JUN 2003
L1         24167 FILE CAPLUS
L2          7371 FILE USPATFULL
     TOTAL FOR ALL FILES
L3         31538 S PDE? OR PHOSPHODIESTERASE?
L4          2656 FILE CAPLUS
L5          4270 FILE USPATFULL
     TOTAL FOR ALL FILES
L6          6926 S (SKIN (5A) HARDEN?) OR SCLERODER?
L7            20 FILE CAPLUS
L8           658 FILE USPATFULL
     TOTAL FOR ALL FILES
L9           678 S L3 AND L6

=> s l8 and (pde2 or pde-2 or pde5 or pde-5)
L10            2 FILE CAPLUS
L11           21 FILE USPATFULL

TOTAL FOR ALL FILES
L12           23 L8 AND (PDE2 OR PDE-2 OR PDE5 OR PDE-5)
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L23 ANSWER 16 OF 18 USPATFULL

DETD A 1.6 kb region of PDE8A(E) encoding the C-terminal 545 amino acids was cloned into the baculovirus transfer vector pFASTBAC, expressed in sf9 cells, and a cell lysate prepared from these cells for enzyme assays. FIG. 6 shows the kinetics of enzyme activity of recombinant, purified PDE8A(E) with cAMP as a substrate. PDE8A(E) has a very high affinity for cAMP with a Km of 55 nM, and a very low affinity for cGMP (Km=124 mM, data not shown). FIG. 7 shows the dependence of PDE8A(E) on divalent cations for maximal activity with a preference for Mn.sup.++ or Mg.sup.++ over Ca.sup.++. The effects of various known PDE inhibitors on the activity of PDE8A(E) are shown in FIG.8. PDE8A(E) was not inhibited by up to 100 mM of rolipram, SKF94120 (inhibitor of PDE3), zaprinast (inhibitor of **PDE5**), vinpocetine (inhibitor of PDE1), of IBMX (non-specific PDE inhibitor). PDE8A(E) was inhibited by dipyridamole (inhibitor of **PDE5**) with an IC.sub.50 of 9 .mu.M. Membrane-based northern analysis shows the expression of this sequence in various tissues, with the most significant expression in testis, ovary, small intestine, and colon.

DETD In another embodiment, an antagonist of PDE8 may be administered to a subject to prevent or **treat** an immune disorder. Such disorders may include, but are not limited to, AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, **scleroderma**, Sjogren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma.

PI US 6080548 20000627

L33 ANSWER 1 OF 61 CAPLUS COPYRIGHT 2003 ACS
AN 2002:905945 CAPLUS
DN 137:389249
TI Medicated stents for the treatment of vascular disease
IN Roorda, Wouter E.; Bhat, Vinayak D.; Mouw, Steven L.; Hossainy, Syed Fa; Wu, Steven Z.; Sanders, Millare Deborra
PA Advanced Cardiovascular Systems, Inc., USA
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61L031-16
ICS A61L031-10
CC 63-7 (Pharmaceuticals)
Section cross-reference(s): 1
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002094335	A1	20021128	WO 2002-US15653	20020514

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI US 2001-860384 A 20010518

AB Stents and bioactive compns. contg. a therapeutic substance for ameliorating a vascular diseased state, particularly for the treatment of stenosis or restenosis following a vascular trauma or disease, are described. The therapeutic substance, e.g., a podophyllotoxin, cephalothin, trapidil, ticlopidine, tranilast, a IIb-IIIa **inhibitor**, clobetasol, a **Cox**-2 **inhibitor**, PGE1, alprostadil, bleomycin, curcumin, **dipyridamole**, tirofiban, verapamil, vitronectin, argatroban, carboplatin, etc., is carried by a polymeric coating supported by the stent. For example, stents coated with EVAL impregnated with actinomycin D induced pos. remodeling of porcine coronary arteries, more particularly pos. remodeling of the external elastic lamina of a blood vessel wall.

ST drug coating stent vascular disease

IT Artery
(angioplasty, vascular trauma assocd. with; medicated stents for treatment of vascular diseases)

IT Artery, disease
(aorta, injury; medicated stents for treatment of vascular diseases)

IT Medical goods
(catheters, administration by; medicated stents for treatment of vascular diseases)

IT Artery
(coronary, injury; medicated stents for treatment of vascular diseases)

IT Prosthetic materials and Prosthetics
(implants, administration by; medicated stents for treatment of vascular diseases)

IT Cell proliferation
(inhibitors; medicated stents for treatment of vascular diseases)

IT Blood vessel, disease
Cardiovascular agents
(medicated stents for treatment of vascular diseases)

IT Vitronectin
RL: DEV (Device component use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(medicated stents for treatment of vascular diseases)
IT Blood vessel, disease
(proliferative; medicated stents for treatment of vascular diseases)
IT Artery, disease
(restenosis; medicated stents for treatment of vascular diseases)
IT Artery, disease
(stenosis; medicated stents for treatment of vascular diseases)
IT Medical goods
(stents; medicated stents for treatment of vascular diseases)
IT Integrins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(.alpha.IIb.beta.3, inhibitors; medicated stents for treatment of vascular diseases)
IT 329900-75-6, **COX** 2
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**inhibitors**; medicated stents for treatment of vascular diseases)
IT 50-35-1, Thalidomide 50-76-0, Actinomycin D 52-53-9, Verapamil
58-32-2, **Dipyridamole** 153-61-7, Cephalotin 362-07-2,
2-Methoxyestradiol 458-37-7, Curcumin 745-65-3, Alprostadil
11056-06-7, Bleomycin 15421-84-8, Trapidil 25067-34-9, Ethylene-vinyl alcohol copolymer 25122-41-2, Clobetasol 29767-20-2, Teniposide
33419-42-0, Etoposide 41575-94-4, Carboplatin 53902-12-8, Tranilast
55142-85-3, Ticlopidine 74863-84-6, Argatroban 86090-08-6, Angiostatin
144494-65-5, Tirofiban 162011-90-7, Rofecoxib 169590-42-5, Celecoxib
187888-07-9, Endostatin 188627-80-7, Eptifibatide
RL: DEV (Device component use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(medicated stents for treatment of vascular diseases)
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Advanced Cardiovascular System; WO 0147572 A 2001 CAPLUS
(2) Advanced Cardiovascular System; WO 0174414 A 2001
(3) Angiogenesis Tech Inc; WO 9503036 A 1995 CAPLUS
(4) Ensovasc Ltd Inc; WO 9834669 A 1998
(5) Ethicon Inc; EP 0970711 A 2000 CAPLUS
(6) Medtronic Inc; EP 1023879 A 2000 CAPLUS
(7) Quanam Medical Corp; WO 0000238 A 2000 CAPLUS
(8) Scimed Life Systems Inc; WO 0062830 A 2000 CAPLUS

L30 ANSWER 3 OF 96 CAPLUS COPYRIGHT 2003 ACS

TI Combination therapy and composition using an antiplatelet agent and a **COX**-2 inhibitor for acute coronary ischemic syndrome and related conditions

AB A method for treating, preventing, or reducing the risk of developing a condition selected from acute coronary ischemic syndrome, thrombosis, thromboembolism, thrombotic occlusion and reocclusion, restenosis, transient ischemic attack, and first or subsequent thrombotic stroke, in a patient comprises administering to the patient a therapeutically effective amt. of an antiplatelet agent in combination with a therapeutically effective amt. of a **COX**-2 inhibitor. The invention also provides a pharmaceutical compn. comprising a therapeutically effective amt. of a **COX**-2 inhibitor, or a pharmaceutically acceptable salt thereof, and an antiplatelet agent, or a pharmaceutically acceptable salt thereof.

ST antiplatelet agent combination acute coronary ischemic syndrome; **COX2** inhibitor combination acute coronary ischemic syndrome; cardiovascular combination cyclooxygenase 2 inhibitor antiplatelet agent

IT 50-78-2, Aspirin 58-32-2, **Dipyridamole** 55142-85-3, Ticlopidine 105806-65-3 105806-65-3D, esters 113665-84-2, Clopidogrel 142373-60-2 142373-60-2D, esters 144412-49-7 144412-49-7D, esters 146144-48-1 146144-48-1D, esters 162011-83-8 162011-90-7 163212-43-9 163212-43-9D, esters 169237-80-3 169237-80-3D, esters 176022-59-6 178402-36-3 185147-73-3 189954-66-3 189954-87-8 189954-93-6 189954-96-9 189956-36-3 190966-03-1 190966-25-7 190966-32-6 202409-31-2 202409-33-4 205385-39-3 205385-39-3D, esters 205385-41-7 205385-41-7D, esters 208260-66-6 208260-66-6D, esters 212126-32-4 223240-38-8 223240-38-8D, esters 223240-39-9 223240-39-9D, esters 223663-01-2 223663-03-4 243637-40-3D, esters

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(antiplatelet agent-cyclooxygenase-2 inhibitor combination for treatment of acute coronary ischemic syndrome and related conditions)

L27 ANSWER 11 OF 239 CAPLUS COPYRIGHT 2003 ACS

AB A pharmaceutical compn. is disclosed for the treatment of neoplasia which comprises a pharmaceutically acceptable carrier and a compd. selected by (1) detg. the cyclooxygenase (**COX**) inhibitory activity of the compd; (2) detg. the phosphodiesterase (**PDE**) inhibition activity of the compd., in which the **PDE** is characterized by (a) cGMP specificity over cAMP, (b) pos. cooperative kinetic behavior in the presence of cGMP substrate, (c) submicromolar affinity for cGMP, and (d) insensitivity to incubation with purified cGMP-dependent protein kinase; and (3) selecting the compd. that has **COX** inhibitory activity lower than the **PDE** activity for treating neoplasia. Also provided is a method for selecting a compd. for the treatment of neoplasia which comprises (1) detg. the **COX** inhibitory activity of the compd.; (2) detg. the **PDE2** inhibition activity of the compd.; and (3) selecting the compd. that has **COX** inhibitory activity lower than the **PDE** activity for treating neoplasia. Isolation of a novel cGMP-specific **PDE** (appearing to be a novel conformation of **PDE2**) from neoplastic cells is described.

L9 ANSWER 19 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 1997:234343 CAPLUS
DN 126:221073
TI Method of promoting angiogenesis and treating wounds using relaxin
IN Unemori, Elaine
PA Connective Therapeutics, Inc., USA
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K038-00
CC 2-4 (Mammalian Hormones)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9706814	A1	19970227	WO 1996-US13321	19960815
	W: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CU, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2229479	AA	19970227	CA 1996-2229479	19960815
	AU 9667771	A1	19970312	AU 1996-67771	19960815
	EP 845992	A1	19980610	EP 1996-928212	19960815
	EP 845992	B1	20021120		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 11511174	T2	19990928	JP 1996-509515	19960815
	US 6211147	B1	20010403	US 1996-698359	19960815
	AT 228004	E	20021215	AT 1996-928212	19960815
	US 2001018418	A1	20010830	US 2001-780758	20010208
PRAI	US 1995-2355P	P	19950815		
	US 1996-698359	A1	19960815		
	WO 1996-US13321	W	19960815		

AB Relaxin is useful for promoting angiogenesis and the treatment of infections or ischemic wounds where the injury results from lack of oxygen due to poor circulation such as in diseases like diabetes or **scleroderma**.
ST relaxin angiogenesis promoter ischemic wound healing
IT Animal cell line
(THP-1; effect of relaxin on VEGF prodn. in THP-1 cells in relation to angiogenesis promotion)
IT Angiogenesis
Anti-infective agents
Diabetes mellitus
Ischemia
Wound healing promoters
(method of promoting angiogenesis and treating infections and ischemic wounds using relaxin)
IT Ulcer
(method of treating ischemic ulcers using relaxin)
IT Transcription, genetic
(relaxin regulation of VEGF transcription in relation to angiogenesis promotion)
IT Connective tissue
(**scleroderma**; method of promoting angiogenesis and treating infections and ischemic wounds using relaxin)
IT 127464-60-2, Vascular endothelial growth factor
RL: BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(effect of relaxin on VEGF prodn. in THP-1 cells in relation to angiogenesis promotion)

IT 9002-69-1, Relaxin 99489-94-8, Human relaxin II
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(method of promoting angiogenesis and treating infections and ischemic wounds using relaxin)

IT 60-92-4, CAMP 9012-42-4, Adenyl cyclase 9036-21-9, CAMP **phosphodiesterase** 141436-78-4, Protein kinase C 142008-29-5, Protein kinase A
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(second messenger pathways in VEGF stimulation by relaxin)

L9 ANSWER 18 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 1997:456086 CAPLUS
DN 127:145194
TI Combined use of angiotensin inhibitors and nitric oxide stimulators to treat fibrosis
IN Chobanian, Aram; Brecher, Peter
PA Trustees of Boston University, USA
SO U.S., 5 pp.
CODEN: USXXAM
DT Patent
LA English
IC ICM A61K009-00
NCL 424400000
CC 1-12 (Pharmacology)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5645839	A	19970708	US 1995-482819	19950607
	US 6139847	A	20001031	US 1997-801512	19970218
PRAI	US 1995-482819	A3	19950607		

AB A combination of angiotensin inhibitors and nitric oxide stimulators is used to slow and reverse the process of fibrosis in the body. This combination of medicaments is particularly useful in the treatment of a variety of cardiovascular fibrotic pathologies, such as that assocd. with left ventricular hypertrophy secondary to hypertension, myocardial infarction, and myocarditis.
ST angiotensin nitric oxide inhibitor combination fibrosis
IT Respiratory distress syndrome
(adult, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Angiotensin receptor antagonists
(angiotensin II; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Antianginal agents
Antiarrhythmics
Anticoagulants
Antihypertensives
Antihypotensives
Diuretics
Fibrosis
Hypolipemic agents
Keloid
Thrombolytics
Vasodilators
(angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Ion channel blockers
(calcium; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Glycosides
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cardiac; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Cardiovascular agents
(cardioplegic; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Cardiovascular system
(disease, fibrosis; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Arteriosclerosis
Cirrhosis
Inflammation
(fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator

combination for fibrosis treatment)
IT Lung, disease
(fibrosis; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Skin, disease
(hypertrophic scar; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Heart, disease
(infarction, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Heart, disease
(left ventricle, hypertrophy, secondary to hypertension, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Hypertension
(left ventricular hypertrophy secondary to, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Fibronectins
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(mRNA; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Heart, disease
(myocarditis, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Resins
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(potassium-removing; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Ion channel openers
(potassium; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Connective tissue
(**scleroderma**, fibrosis assocd. with; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Collagens, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(type III, mRNA; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Adrenoceptor antagonists
(.alpha.-; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT Adrenoceptor antagonists
(.beta.-; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT 74-79-3, L-Arginine, biological studies 50903-99-6, L-NAME
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT 114798-26-4, Losartan
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)
IT 55-63-0, Nitroglycerin 78-11-5, Pentaerythritol tetranitrate 87-33-2, Isosorbide dinitrate 139-33-3, Disodium edetate 1002-16-0, Amyl nitrate 15078-28-1, Nitroprusside 62571-86-2, Captopril 74258-86-9, Alacepril 75847-73-3, Enalapril 76420-72-9, Enalaprilat 76547-98-3, Lisinopril 80830-42-8, Rentiapril 81872-10-8, Zofenopril 82834-16-0, Perindopril 82924-03-6, Pentopril 83435-66-9, Delapril 83647-97-6,

Spirapril 85441-61-8, Quinapril 87333-19-5, Ramipril 88768-40-5, Cilazapril 98048-97-6, Fosinopril 111223-26-8, Ceranapril
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 11128-99-7, Angiotensin II
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(antagonists and catabolism activators; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 7440-09-7, Potassium, biological studies
RL: BSU (Biological study, unclassified); REM (Removal or disposal); BIOL (Biological study); PROC (Process)
(channel, activators, and potassium-removing resins; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 7440-70-2, Calcium, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(channel, blockers; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 1407-47-2, Angiotensin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibitors; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 9015-82-1, Angiotensin-converting enzyme 9015-82-1 9025-82-5, **Phosphodiesterase** 9041-90-1, Angiotensin I
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitors; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 85637-73-6, Atrial natriuretic factor
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(mRNA; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 10102-43-9, Nitric oxide, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(stimulators; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

IT 125978-95-2, Nitric oxide synthase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(stimulators; angiotensin inhibitor-nitric oxide stimulator combination for fibrosis treatment)

L9 ANSWER 16 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 1999:27702 CAPLUS
DN 130:76179
TI Use of nitric oxide for treatment of erectile dysfunction
IN Adams, Michael A.; Heaton, Jeremy P. W.; Banting, James D.
PA Queen's University at Kingston, Can.
SO PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K031-00
CC 1-8 (Pharmacology)
Section cross-reference(s): 63
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9858633	A2	19981230	WO 1998-CA603	19980622
	WO 9858633	A3	19990812		
	W: AU, BR, CA, JP, MX, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9880961	A1	19990104	AU 1998-80961	19980622
	AU 756136	B2	20030102		
	EP 998274	A2	20000510	EP 1998-930577	19980622
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002505676	T2	20020219	JP 1999-503464	19980622
	MX 9911610	A	20000630	MX 1999-11610	19991213
	US 6165975	A	20001226	US 1999-469649	19991222
	US 6423683	B1	20020723	US 2000-613637	20000711
	US 2002091088	A1	20020711	US 2002-95654	20020308
PRAI	US 1997-50491P	P	19970623		
	US 1998-86750P	P	19980527		
	WO 1998-CA603	W	19980622		
	US 1999-469649	A1	19991222		
	US 2000-613637	A1	20000711		

AB Methods for treating vascular conditions assocd. with localized imbalance in vascular tone, which are hypothesized to be largely due to elevated endothelin (ET), are provided. The methods involve administration of nitric oxide (NO), agents which are able to provide NO, such as NO donors, agents which activate guanyl cyclase, such as YC-1, or agents which prolong the actions of endogenous NO or cyclic guanosine monophosphate (cGMP; a 2nd messenger mol.), such as **phosphodiesterase** (**PDE**) inhibitors. According to the invention, such agents are administered in minimal doses or microdoses by any route known in the art, so as to provide dosages which are about one half to about one twentieth of those known to induce vasodilation in "normal" circulations. The low doses of these agents effectively alleviate vascular conditions assocd. with a redn. in NO prodn. or an attenuation of NO effect, by restoring balance in vascular tone while exerting almost no systemic effect in normal vasculature. A patient with a total erectile dysfunction of 18 mo was prescribed 0.2 mg/h patch contg. glyceryl trinitrate with no effects on systemic blood pressure and 100% successful intercourse.

ST nitric oxide erectile dysfunction pharmaceutical

IT Blood vessel, disease
(Raynaud's phenomenon; use of nitric oxide for treatment of erectile dysfunction)

IT Drug delivery systems
(buccal; use of nitric oxide for treatment of erectile dysfunction)

IT Estrogens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(depletion conditions; use of nitric oxide for treatment of erectile dysfunction)

IT Sexual behavior

(disorder; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(enteric; use of nitric oxide for treatment of erectile dysfunction)
IT Vagina
(idiosyncratic dryness of; use of nitric oxide for treatment of erectile dysfunction)
IT Sexual behavior
(impotence; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(inhalants; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(injections, i.v.; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(oral; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(parenterals; use of nitric oxide for treatment of erectile dysfunction)
IT Prostate gland
Prostate gland
(prostatectomy; suse of nitric oxide for treatment of erectile dysfunction)
IT Connective tissue
(**scleroderma**; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(sublingual; use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(transdermal; use of nitric oxide for treatment of erectile dysfunction)
IT Infection
(urogenital; use of nitric oxide for treatment of erectile dysfunction)
IT Blood vessel, disease
Brain
Diabetes mellitus
Digestive tract
Kidney
Muscle
Penis
Skin
Vasodilators
(use of nitric oxide for treatment of erectile dysfunction)
IT Drug delivery systems
(vaginal; use of nitric oxide for treatment of erectile dysfunction)
IT 9054-75-5, Guanyl cyclase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(activator; use of nitric oxide for treatment of erectile dysfunction)
IT 9025-82-5, **Phosphodiesterase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(inhibitors; use of nitric oxide for treatment of erectile dysfunction)
IT 55-63-0, Glyceryl trinitrate 78-11-5, Pentaerythritoltetranitrate 87-33-2, Isosorbide dinitrate 630-08-0, Carbon monoxide, biological studies 7297-25-8, Erythrityl tetranitrate 10102-43-9, Nitric oxide, biological studies 14402-89-2, Sodium nitroprusside 16051-77-7, Isosorbide 5-mononitrate 25717-80-0 53054-07-2 57564-91-7, S-Nitrosoglutathione 79032-48-7, s-Nitroso-N-acetylpenicillamine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(use of nitric oxide for treatment of erectile dysfunction)
IT 7665-99-8, Cyclic guanosine monophosphate 116243-73-3, Endothelin 170632-47-0, YC-1
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(use of nitric oxide for treatment of erectile dysfunction)

L9 ANSWER 11 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 2002:31259 CAPLUS
DN 136:64173
TI Method using sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treating peripheral vascular diseases, peripheral neuropathies,and autonomic neuropathies
IN Wood, Ralph E.
PA USA
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K031-495
CC 1-12 (Pharmacology)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002002118	A1	20020110	WO 2001-US41202	20010629
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1303279	A1	20030423	EP 2001-957540	20010629
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	BR 2001012100	A	20030520	BR 2001-12100	20010629
	US 2003105108	A1	20030605	US 2002-311907	20021219
PRAI	US 2000-215065P	P	20000630		
	US 2000-219029P	P	20000718		
	WO 2001-US41202	W	20010629		

AB A method is provided for treating a patient suffering from peripheral vascular disease, peripheral neuropathies, or autonomic neuropathies by administering a cGMP **PDE5** inhibitor such as sildenafil. The method is particularly applicable to patients suffering from diabetic foot ulcers, Raynaud's Phenomenon, CREST Syndrome, erythromatosis, rheumatoid diseases, diabetic retinopathies and onychomycosis. According to the invention, a cGMP **PDE5** inhibitor may be administered as a prophylactic to patients predisposed to develop a peripheral vascular disease, peripheral neuropathy, or autonomic neuropathy.

ST vascular peripheral disease cGMP **phosphodiesterase** 5 inhibitor; peripheral autonomic neuropathy cGMP **phosphodiesterase** 5 inhibitor; sildenafil vascular peripheral disease peripheral autonomic neuropathy

IT Blood vessel, disease
(Raynaud's phenomenon; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

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(autonomic neuropathy; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Antiulcer agents
Diabetes mellitus
Foot
(diabetic foot ulcer; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Eye, disease
(diabetic retinopathy; sildenafil or other cGMP

phosphodiesterase 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Disease, animal
(erythromatosis; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Nail (anatomical), disease
(onychomycosis; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

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(rheumatoid disease; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Connective tissue, disease
(**scleroderma**, CREST syndrome variant; sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT Drug delivery systems
Fungicides
(sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT 9068-52-4, **Phosphodiesterase** V
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

IT 139755-83-2, Sildenafil
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Bombrun; US 6043252 A 2000 CAPLUS
(2) Graham; US 6075028 A 2000 CAPLUS

L9 ANSWER 11 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 2002:31259 CAPLUS
DN 136:64173
TI Method using sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treating peripheral vascular diseases, peripheral neuropathies,and autonomic neuropathies
IN Wood, Ralph E.
PA USA
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2
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CC 1-12 (Pharmacology)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002002118	A1	20020110	WO 2001-US41202	20010629
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1303279	A1	20030423	EP 2001-957540	20010629
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	BR 2001012100	A	20030520	BR 2001-12100	20010629
	US 2003105108	A1	20030605	US 2002-311907	20021219
PRAI	US 2000-215065P	P	20000630		
	US 2000-219029P	P	20000718		
	WO 2001-US41202	W	20010629		

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IT 9068-52-4, **Phosphodiesterase** V
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IT 139755-83-2, Sildenafil
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treatment of peripheral vascular diseases and peripheral and autonomic neuropathies)

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Bombrun; US 6043252 A 2000 CAPLUS
(2) Graham; US 6075028 A 2000 CAPLUS

L9 ANSWER 11 OF 678 CAPLUS COPYRIGHT 2003 ACS
AN 2002:31259 CAPLUS
DN 136:64173
TI Method using sildenafil or other cGMP **phosphodiesterase** 5 inhibitor for treating peripheral vascular diseases, peripheral neuropathies,and autonomic neuropathies
IN Wood, Ralph E.
PA USA
SO PCT Int. Appl., 16 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K031-495
CC 1-12 (Pharmacology)
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002002118	A1	20020110	WO 2001-US41202	20010629
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	EP 1303279	A1	20030423	EP 2001-957540	20010629
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
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	US 2000-219029P	P	20000718		
	WO 2001-US41202	W	20010629		

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IT 139755-83-2, Sildenafil
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
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RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Bombrun; US 6043252 A 2000 CAPLUS
(2) Graham; US 6075028 A 2000 CAPLUS

L23 ANSWER 18 OF 18 USPATFULL

DETD A 1.8 kb region of PDE9A encoding the full length of the protein was cloned into the baculovirus transfer vector pFASTBAC, expressed in sf9 cells, and a cell lysate prepared from these cells for enzyme assays. FIG. 3 shows the kinetics of enzyme activity of recombinant, purified PDE9A with cGMP as a substrate. PDE9A has a very high affinity for cGMP with a Km of 170 nM, and a very low affinity for cAMP (Km=230 .upsilon.M, data not shown). FIG. 4 shows the dependence of PDE9A on divalent cations for maximal activity with a preference for Mn.sup.++ over Mg.sup.++ or Ca.sup.++. The effects of various known PDE inhibitors on the activity of PDE9A are shown in FIG. 5. PDE9A was not inhibited by up to 100 .mu.M of rolipram (inhibitor of PDE4), dipyridamole (inhibitor of **PDE2**, 4, 5, and 6), SKF94120 (inhibitor of PDE3), vinpocetine (inhibitor of PDE1), or IBMX (non-specific PDE inhibitor). PDE9A was inhibited by zaprinast (inhibitor of **PDE5** and 6) with an IC.sub.50 of 35 .mu.M. Membrane-based northern analysis shows the expression of this sequence in various tissues, with the most significant expression in testis, ovary, small intestine, and colon. Electronic northern analysis using the LIFESEQ database further shows the expression of this sequence in various tissues, at least 50% of which are cancerous and at least 25% of which involves inflammation or the immune response. Of particular note is the expression of PDE9A in Crohn's disease.

DETD In another embodiment, an antagonist of PDE9A may be administered to a subject to prevent or **treat** an immune disorder. Such disorders may include, but are not limited to, AIDS, Addison's disease, adult respiratory distress syndrome, allergies, anemia, asthma, atherosclerosis, bronchitis, cholecystitis, Crohn's disease, ulcerative colitis, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythema nodosum, atrophic gastritis, glomerulonephritis, gout, Graves' disease, hypereosinophilia, irritable bowel syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, rheumatoid arthritis, **scleroderma**, Sjogren's syndrome, and autoimmune thyroiditis; complications of cancer, hemodialysis, extracorporeal circulation; viral, bacterial, fungal, parasitic, protozoal, and helminthic infections; and trauma.

PI US 5922595 19990713

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L23 ANSWER 1 OF 2 USPATFULL
ACCESSION NUMBER: 2002:16884 USPATFULL
TITLE: METHODS FOR IDENTIFYING COMPOUNDS FOR INHIBITION OF NEOPLASTIC LESIONS, AND PHARMACEUTICAL COMPOSITIONS CONTAINING SUCH COMPOUNDS
INVENTOR(S): THOMPSON, W. JOSEPH, DOYLESTOWN, PA, UNITED STATES
LIU, LI, AMBLER, PA, UNITED STATES
LI, HAN, YARDLEY, PA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002009764	A1	20020124	
APPLICATION INFO.:	US 1999-414628	A1	19991008	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ROBERT W STEVENSON, CELL PATHWAYS INC, 702 ELECTRONIC DR, HORSHAM, PA, 10944
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 25 Drawing Page(s)
LINE COUNT: 2468
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 2 OF 2 USPATFULL
ACCESSION NUMBER: 2000:80548 USPATFULL
TITLE: Cloning of mammalian genes in microbial organisms and methods for pharmacological screening
INVENTOR(S): Wigler, Michael H., Lloyd Harbor, NY, United States
Colicelli, John J., Huntington, NY, United States
PATENT ASSIGNEE(S): Cold Spring Harbor Laboratory, Cold Spring, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6080540		20000627	
APPLICATION INFO.:	US 1990-511715		19900420	(7)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Guzo, David
LEGAL REPRESENTATIVE: Marshall, O'Toole, Gerstein, Murray & Borun
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT: 1177
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=>

L6 ANSWER 1 OF 16 USPATFULL

ACCESSION NUMBER:	2002:224620 USPATFULL
TITLE:	Methods and compositions for treating Raynaud's Phenomenon and scleroderma
INVENTOR(S):	Flavahan, Nicholas, Columbus, OH, United States Flavahan, Sheila, Columbus, OH, United States Chotani, Maqsood, Columbus, OH, United States Mitra, Srabani, Worthington, OH, United States Su, Baogen, Columbus, OH, United States
PATENT ASSIGNEE(S):	The Ohio State University Research Foundation, Columbus, OH, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6444681	B1	20020903	
APPLICATION INFO.:	US 2000-591254		20000609	(9)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Fay, Zohreh
LEGAL REPRESENTATIVE:	Calfee, Halter & Griswold LLP
NUMBER OF CLAIMS:	18
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	10 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT:	798

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for treating conditions or diseases associated with deleterious vasoconstriction of the small arteries and arterioles of one or more organs or parts of a patient's body. In one embodiment, the method comprises administering a therapeutically effective amount of an antagonist to the .alpha..sub.2C-adrenergic receptor (.alpha..sub.2C-AR) to a patient with Raynaud's Phenomenon. Such method is used to ameliorate the cold-induced or stress-induced vasoreactive response that is associated with Raynaud's Phenomenon. The .alpha..sub.2C-AR antagonist is administered to the subject either prior to or after exposure of the patient to the cold or to stressIn another embodiment, the method is used to reduce the extent of deleterious vasoconstriction that occurs in the small arteries, arterioles, and microcirculation of the lungs, heart, kidneys, skin, or gastrointestinal tract of a patient, particularly a **scleroderma** patient. The method comprises administering a therapeutically effective amount of an .alpha..sub.2C-AR antagonist to a patient who is in need of the same. Such treatment serves to maintain or restore, at least in part, blood flow through the small arteries, arterioles, and microcirculation of the lungs, heart, kidneys, skin, and/or gastrointestinal tract in a such patient. The present invention also relates to a pharmaceutical compositions comprising an .alpha..sub.2C-AR antagonist and a pharmaceutically acceptable carrier.

CLM What is claimed is:

1. A method of treating a patient with a condition that involves vasoconstriction of the small arteries or arterioles of a part or organ of the patient's body, comprising: administering to the patient a therapeutically effective amount of an .alpha..sub.2C receptor antagonist that selectively binds to an .alpha..sub.2C adrenergic receptor.

2. The method of claim 1 wherein the antagonist is a reversible .alpha..sub.2C adrenergic receptor antagonist.

3. The method of claim 1 wherein the wherein the antagonist is administered prior to exposure of the subject to cold or stress.

4. The method of claim 1 wherein the antagonist is administered after exposure of the subject to cold or stress.

5. The method of claim 1 wherein the antagonist is administered in an oral composition or a topical composition.

6. The method of claim 1 wherein the antagonist is administered in an amount sufficient to increase blood flow through the small arteries or arterioles of the affected organ.

7. The method of claim 1 wherein the patient is exhibiting symptoms of Raynaud's phenomenon.

8. The method of claim 1 wherein the patient is exhibiting symptoms of ischemia of the small arteries or arterioles of an organ selected from the group consisting of kidney, heart, lungs, gastrointestinal tract and combinations thereof.

9. The method of claim 8 wherein the patient has **scleroderma**.

10. The method of claim 8 wherein the antagonist is administered to the patient in a pharmaceutical composition that is orally ingested or inhaled by the patient or injected into the patient.

11. The method of claim 8 wherein the amount of antagonist administered is from 0.01 .mu.g to about 100 mg of antagonist per kg of body weight.

12. The method of claim 8 wherein the antagonist is administered in multiple doses.

13. A method of reducing cold-induced vasoconstriction of a small cutaneous artery or arteriole in at least one organ or part of a patient's body, comprising contacting the vascular smooth muscle cells of said artery or said arteriole with an .alpha..sub.2C receptor antagonist that selectively binds to an .alpha..sub.2C adrenergic receptor.

14. A method of reducing constriction of the small arteries or arterioles in an organ selected from the group consisting of heart, lung, kidney, gastrointestinal tract and combinations thereof comprising contacting the vascular smooth muscle cells of said artery or arteriole with an .alpha..sub.2C receptor antagonist that selectively binds to an .alpha..sub.2C adrenergic receptor.

15. The method of claim 1, wherein the antagonist is a competitive inhibitor of an .alpha.2 adrenergic receptor agonist.

16. The method of claim 13, wherein the antagonist is a competitive inhibitor of an .alpha.2 adrenergic receptor agonist.

17. The method of claim 14, wherein the antagonist is a competitive inhibitor of an .alpha.2 adrenergic receptor agonist.

18. The method of claim 13, wherein the antagonist is a competitive inhibitor of an .alpha.2 adrenergic receptor agonist.

L6 ANSWER 2 OF 16 USPATFULL
ACCESSION NUMBER: 2002:191186 USPATFULL
TITLE: Attenuation of fibroblast proliferation
INVENTOR(S): Denholm, Elizabeth M., Pointe Claire, CANADA
Cauchon, Elizabeth, Ile Perrot, CANADA
Silver, Paul J., Spring City, PA, UNITED STATES
PATENT ASSIGNEE(S): IBEX Technologies (non-U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION:	US 2002102249	A1	20020801
APPLICATION INFO.:	US 2000-727873	A1	20001201 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-168518P	19991202 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Patrea L. Pabst, Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA, 30309-3450
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Page(s)
LINE COUNT: 765
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Highly purified and specific glycosaminoglycan degrading enzymes, chondroitinase B and chondroitinase AC, are used to treat fibroproliferative diseases. The enzymatic removal of chondroitin sulfate B (dermatan sulfate), and to a lesser extent, chondroitin sulfate A or C, from cell surfaces effectively decreases growth factor receptors on the cells and thereby decreases the cell proliferative response to such growth factors. In addition, removal of chondroitin sulfates reduces secretion of collagen, one of the major extracellular matrix components. Through the combined inhibition of fibroblast proliferation and collagen synthesis, treatment with chondroitinase B or chondroitinase AC decreases the size of fibrous tissue found in psoriasis, **scleroderma**, keloids, pulmonary fibrosis and surgical adhesions.

CLM What is claimed is:

1. A method to modulate fibrous tissue formation comprising administering to an individual in need of treatment thereof an effective amount of a dematan sulfate or chondroitin sulfate degrading enzyme.

2. The method of claim 1 wherein the enzyme is selected from the group consisting of bacterial dermatan or chondroitin sulfate degrading enzyme and is selected from the group consisting of chondroitinase AC from Flavobacterium heparinum, chondroitinase B from Flavobacterium heparinum, chondroitin sulfate degrading enzymes from Bacteroides species, chondroitin sulfate degrading enzymes from Proteus vulgaris, chondroitin sulfate degrading enzymes from Micrococcus, chondroitin sulfate degrading enzymes from Vibrio species, chondroitin sulfate degrading enzymes from Arthrobacter aurescens, arylsulfatase B, N-acetylgalactosamine-6-sulfatase and iduronate sulfatase from mammalian cells, these enzymes expressed from recombinant nucleotide sequences in bacteria and combinations thereof.

3. The method of claim 1 wherein the enzyme is a mammalian enzyme.

4. The method of claim 1 wherein the enzyme is a bacterial enzyme.

5. The method of claim 4 wherein the chondroitinase is chondroitinase B.

6. The method of claim 1 wherein the individual has a skin disorder.

7. The method of claim 6 wherein the skin disorder is **scleroderma** or psoriasis.

8. The method of claim 1 wherein the individual has keloid scarring or is at risk of keloid scarring, or has pulmonary fibrosis.

9. The method of claim 1 wherein the enzyme is administered systemically.

10. The method of claim 1 wherein the enzyme is administered topically or locally at or adjacent to a site in need of treatment.

11. The method of claim 1 wherein the enzyme is administered in a controlled and/or sustained release formulation.

12. A formulation for administration to an individual in need of treatment thereof for a disorder involving organ fibrosis, the formulation comprising an effective amount of a dermatan or chondroitin sulfate degrading enzyme to inhibit fibrosis, wherein the dosage is different than the amount effective for wound healing, and a pharmaceutically acceptable carrier.

13. The formulation of claim 12 wherein the enzyme is selected from the group consisting of bacterial chondroitin sulfate degrading enzyme and is selected from the group consisting of chondroitinase AC from Flavobacterium heparinum, chondroitinase B from Flavobacterium heparinum, chondroitin sulfate degrading enzymes from Bacteroides species, chondroitin sulfate degrading enzymes from Proteus vulgaris, chondroitin sulfate degrading enzymes from Micrococcus, chondroitin sulfate degrading enzymes from Vibrio species, chondroitin sulfate degrading enzymes from Arthrobacter aurescens, these enzymes expressed from recombinant nucleotide sequences in bacteria and combinations thereof.

14. The formulation of claim 12 wherein the enzyme is a mammalian enzyme.

15. The formulation of claim 12 wherein the enzyme is a bacterial chondroitinase.

16. The formulation of claim 15 wherein the chondroitinase is chondroitinase B.

17. The formulation of claim 12 wherein the enzyme is in a controlled, sustained release formulation.

18. The formulation of claim 12 in a dosage effective to collagen synthesis.

19. The formulation of claim 12 in an effective aerosol formulation for delivery to the lungs.

L6 ANSWER 3 OF 16 USPATFULL

ACCESSION NUMBER:	2002:48655 USPATFULL
TITLE:	Method for treatment of fibrosis related diseases by the administration of prostacyclin derivatives
INVENTOR(S):	Stratton, Richard, London, UNITED KINGDOM Black, Carol, London, UNITED KINGDOM Martin, George R., Palo Alto, CA, UNITED STATES Carmichael, David F., Pacifica, CA, UNITED STATES
PATENT ASSIGNEE(S):	Fibrogen, Inc. (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002028847	A1	20020307
APPLICATION INFO.:	US 2001-915947	A1	20010725 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-349516, filed on 8 Jul 1999, ABANDONED

NUMBER	DATE

PRIORITY INFORMATION: US 1998-92044P 19980708 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ROBINS & PASTERNAK LLP, Suite 200, 90 Middlefield Road, Menlo Park, CA, 94025
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Page(s)
LINE COUNT: 640
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to methods for treating fibrosis related diseases and disorders, particularly **scleroderma** by treating a patient in need with a pharmaceutically efficacious amount of a.prostacyclin derivative. The most preferred prostacyclin derivatives are cicaprost and iloprost.

CLM What is claimed is:

1. A method for treating a fibrotic disorder comprising administering to a patient in need a therapeutically effective amount of a prostacyclin derivative.

2. The method of claim 1 wherein said fibrotic disorder is **scleroderma**.

3. The method of claim 1 wherein said prostacyclin derivative is cicaprost.

4. The method of claim 1 wherein said prostacyclin derivative is iloprost.

5. A method for ameliorating the fibrosis related symptoms of **scleroderma** comprising administering to a patient in need a therapeutically effective amount of a prostacyclin derivative.

6. The method of claim 7 wherein said prostacyclin derivative is cicaprost.

7. The method of claim 7 wherein said prostacyclin derivative is iloprost.

L6 ANSWER 4 OF 16 USPATFULL
ACCESSION NUMBER: 2001:224167 USPATFULL
TITLE: Method of treatment and pharmaceutical composition
INVENTOR(S): Webb, Randy Lee, Flemington, NJ, United States
Gasparo, Marc de, Es Planches, Switzerland

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001049384	A1	20011206
	US 6395728	B2	20020528
APPLICATION INFO.:	US 2001-757413	A1	20010109 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1999-349654, filed on 8 Jul 1999, GRANTED, Pat. No. US 6204281
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: THOMAS HOXIE, NOVARTIS CORPORATION, PATENT AND TRADEMARK DEPT, 564 MORRIS AVENUE, SUMMIT, NJ, 079011027
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
LINE COUNT: 416
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension,

(acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, myocardial infarction and its sequelae, supraventricular and ventricular arrhythmias, atrial fibrillation or atrial flutter, atherosclerosis, angina (whether stable or ustable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetessecondary aldosteronism, primary and secondary pulmonary hyperaldosteronism, primary and pulmonary hypertension, renal failure conditions, such as diabetic nephropathy,glomerulonephritis, **scleroderma**, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), and stroke, comprising administering a therapeutically effective amount of combination of (i) the AT.sub.1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a Calcium channel blocker or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment and to corresponding pharmaceutical combination composition.

CLM What is claimed is:

1. A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, myocardial infarction and its sequelae, supraventricular and ventricular arrhythmias, atrial fibrillation or atrial flutter, atherosclerosis, angina (whether stable or ustable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetessecondary aldosteronism, primary and secondary pulmonary hyperaldosteronism, primary and pulmonary hypertension, renal failure conditions, diabetic nephropathy,glomerulonephritis, **scleroderma** , glomerular sclerosis, proteinuria of primary renal disease, renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, migraine, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, Alzheimer's, and stroke, comprising administering a therapeutically effective amount of combination of (i) the AT.sub.1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a Calcium channel blocker or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

2. A method as claimed in claim 1, wherein a Calcium channel blocker selected from the group consisting of amlodipine, diltiazem, felodipine, fendiline, flunarizine, gallopamil, isradipine, lacidipine, mibefradil, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, nivaldipine, ryosidine, tiapamil and verapamil, and in each case, a pharmaceutically acceptable salt thereof is used as component (ii).

3. A method as claimed in claim 1, wherein amlodipine or a pharmaceutically acceptable salt thereof is used as component (ii).

4. A pharmaceutical combination composition comprising (i) the AT.sub.1-antagonists valsartan or a pharmaceutically acceptable salt thereof and (ii) a Calcium channel blocker or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

5. A pharmaceutical combination composition as claimed in claim 4 for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation or atrial flutter, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina

pectoris, diabetessecondary aidosteronism, primary and secondary pulmonary hyperaldosteronism, primary and pulmonary hypertension, renal failure conditions, diabetic nephropathy, glomerulonephritis, **scleroderma**, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, migraine, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, Alzheimer's, and stroke.

6. A pharmaceutical combination composition as claimed in claim 4, comprising a Calcium channel blocker selected from the group consisting of amlodipine, diltiazem, felodipine, fendiline, flunarizine, gallopamil, isradipine, lacidipine, mibefradil, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, nivaldipine, ryosidine, tiapamil and verapamil, or in each case, a pharmaceutically acceptable salt thereof.

7. A pharmaceutical combination composition as claimed in claim 4, comprising amlodipine or a pharmaceutically acceptable salt thereof.

8. A pharmaceutical combination composition as claimed in claim 4 for oral application, comprising of about 10 mg to about 200 mg of valsartan.

9. A pharmaceutical combination composition as claimed in claim 4 for oral application, comprising about 1.0 mg to about 180 mg of a Calcium channel blocker.

L6 ANSWER 5 OF 16 USPATFULL

ACCESSION NUMBER:	2001:105360 USPATFULL		
TITLE:	METHOD FOR TREATMENT OF FIBROSIS RELATED DISEASES BY THE ADMINISTRATION OF PROSTACYCLIN DERIVATIVES		
INVENTOR(S):	STRATTON, RICHARD, LONDON, Great Britain BLACK, CAROL M., LONDON, Great Britain MARTIN, GEORGE R., PALO ALTO, CA, United States CARMICHAEL, DAVID F., PACIFICA, CA, United States		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001006979	A1	20010705
APPLICATION INFO.:	US 1999-349516	A1	19990708 (9)
	NUMBER		DATE
PRIORITY INFORMATION:	US 1998-92044P		19980708 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	LEGAL DEPARTMENT, FIBROGEN INC, 225 GATEWAY BLVD, SOUTH SAN FRANCISCO, CA, 94080		
NUMBER OF CLAIMS:	7		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	2 Drawing Page(s)		
LINE COUNT:	630		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to methods for treating fibrosis related diseases and disorders, particularly **scleroderma** by treating a patient in need with a pharmaceutically efficacious amount of a prostacyclin derivative. The most preferred prostacyclin derivatives are cicaprost and iloprost.

CLM What is claimed is:

1. A method for treating a fibrotic disorder comprising administering to a patient in need a therapeutically effective amount of a prostacyclin derivative.

2. The method of claim 1 wherein said fibrotic disorder is **scleroderma**.

3. The method of claim 1 wherein said prostacyclin derivative is cicaprost.

4. The method of claim 1 wherein said prostacyclin derivative is iloprost.

5. A method for ameliorating the fibrosis related symptoms of **scleroderma** comprising administering to a patient in need a therapeutically effective amount of a prostacyclin derivative.

6. The method of clam 7 wherein said prostacyclin derivative is cicaprost.

7. The method of claim 7 wherein said prostacyclin derivative is iloprost.

L6 ANSWER 6 OF 16 USPATFULL

ACCESSION NUMBER:	2001:59865 USPATFULL			
TITLE:	Antifungal peptides from scleroderma texense			
INVENTOR(S):	Stump, Heike, Karben, Germany, Federal Republic of Stahl, Wilhelm, Idstein, Germany, Federal Republic of Wink, Joachim, Rodermark, Germany, Federal Republic of Markus, Astrid, Liederbach, Germany, Federal Republic of Kogler, Herbert, Glashutten, Germany, Federal Republic of Backhaus, Jurgen, Edingen, Germany, Federal Republic of			
PATENT ASSIGNEE(S):	Aventis Pharma Deutschland GmbH, Frankurt am Main, Germany, Federal Republic of (non-U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6221844	B1	20010424	
	WO 9736921		19971009	
APPLICATION INFO.:	US 1999-155603		19990518	(9)
	WO 1997-EP1507		19970325	
			19990518	PCT 371 date
			19990518	PCT 102(e) date
	NUMBER		DATE	
PRIORITY INFORMATION:	DE 1996-19612805		19960401	
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Davenport, Avis M.			
LEGAL REPRESENTATIVE:	Foley & Lardner			
NUMBER OF CLAIMS:	14			
EXEMPLARY CLAIM:	1			
LINE COUNT:	274			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to novel peptides, namely texenomycins, from **Scleroderma** texense, a process for their preparation and their use as pharmaceuticals, in particular as antimycotics.

The texenomycins according to the present invention are distinguished in particular by the amino acid sequence I ##STR1##

in which

FS is an oxo fatty acid radical,

Aib is aminoisobutyric acid and

Arg-ol=argininol.

CLM What is claimed is:

1. A peptide of amino acid sequence I (SEQ ID NO: 1) ##STR4## in which FS is an oxo fatty acid radical, Aib is aminoisobutyric acid and Arg-ol=argininol.

2. A peptide as claimed in claim 1, wherein the oxo fatty acid radical consists of 3 to 20 carbon atoms.

3. A peptide as claimed in claim 2, wherein the oxo fatty acid radical consists of 8 to 15 carbon atoms.

4. A peptide as claimed in claim 3, wherein the oxo group of the oxo fatty acid radical is found in the 3-position.

5. A peptide as claimed in claim 4, wherein the oxo fatty acid radical has the constitutional formula II ##STR5##

6. A peptide as claimed in claim 5, wherein the fatty acid radical has an R configuration.

7. A peptide as claimed in claim 5, wherein the fatty acid radical has an S configuration.

8. A process for the preparation of a compound as claimed in claim 1, which comprises culturing **Scleroderma** texense in an aqueous medium and then isolating and purifying the target compound.

9. The process as claimed in claim 8, wherein **Scleroderma** texense DSM 10601 is employed.

10. The process as claimed in claim 9, wherein mutants or variants of **Scleroderma** texense DSM 10601 are employed.

11. A peptide as claimed in claim 1 for use as a pharmaceutical.

12. A pharmaceutical comprising at least one peptide as claimed in claim 1 and pharmaceutical auxiliaries.

13. **Scleroderma** texense DSM 10601 and its mutants and variants.

14. A method of treating fungal infections, comprising administering the peptide of claim 1 to an individual in need thereof.

L6 ANSWER 7 OF 16 USPATFULL

ACCESSION NUMBER: 1999:78760 USPATFULL

TITLE: Immunomodulator, cell adhesion inhibtor, and agent for treating, and preventing autoimmune diseases

INVENTOR(S): Tanaka, Keiichi, Toyama, Japan
Makino, Shinji, Kurobe, Japan
Oshio, Ichiro, Machida, Japan
Shimotori, Tomoya, Toyama, Japan
Aikawa, Yukihiko, Toyama, Japan
Inaba, Takihiro, Namerikawa, Japan
Yoshida, Chosaku, Takaoka, Japan
Takano, Shuntaro, Mitaka, Japan
Taniguchi, Yoichi, Takaoka, Japan

PATENT ASSIGNEE(S): Toyama Chemical Co., Ltd., Tokyo, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5922755		19990713	
	WO 9423714		19941027	
APPLICATION INFO.:	US 1996-530177		19960506	(8)
	WO 1994-JP585		19940407	
			19960506	PCT 371 date
			19960506	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1993-107464	19930409

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Criares, Theodore J.
LEGAL REPRESENTATIVE: Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
LINE COUNT: 751
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A 4H-1-benzopyran-4-one derivative of the general formura [1], ##STR1## or a salt thereof exerts excellent effects on immunomodulation and cell adhesion inhibition, and is further expected to have the effect of relieving autoimmune diseases at a level comparable to that of steroids. Thus, the compound of the general formula [1] is useful in the teatment and prevention of autoimmune diseases fundamentally caused by immunopathy or unusually accelerated cell adhesion, for example, chronic rheumatoid arthritis, systemic lupus erythematosus, **scleroderma**, mixed connective tissue disease,polyarteritis nodosa, polymyositis/dermatomyositis, Sjogren's syndrome, Behcet's disease, multiple sclerosis, autoimmune diabetes, Hashimoto's disease, psoriasis, primary myxedema, pernicious anemia, serious adynamia, ulcerative colitis, chronic active hepatitis, autoimmune hemolytic anemia idiopathic thrombocytopenic purpura and the like.

CLM What is claimed is:

1. A method of treating an individual suffering from an autoimmune disease mediated by cell adhesion, comprising: administering to a patient in need of same a a cell adhesion inhibiting amount of a 4H-1-benzopyran4-one compound represented by the following formula or a salt thereof: ##STR4## wherein R.sup.1 is an unsubstituted or halogen-substituted alkyl, alkenyl or aryl group; R.sup.2 is a hydrogen atom or an alkyl or acyl group; R.sup.3 is a hydrogen or halogen atom or a cyano, azido, carboxyl, hydroxyl, formyl or alkoxycarbonyl group or a substituted or unsubstituted alkyl, alkoxy, phenoxy, cycloalkyl, carbamoyl, amino or phenyl group; R.sup.4 is a hydrogen or halogen atom, a nitro, cyano, carboxyl, acyl, hydroxyl or alkoxycarbonyl group, or a substituted or unsubstituted alkyl, alkoxy, alkylthio, phenylthio, alkynyl, alkenyl, sulfamoyl, alkanesulfinyl, alkanesulfonyl, amidino, phenyl or heterocyclic group or a group of the formula ##STR5## where R.sup.6 is a hydrogen atom, a hydroxyl, cyano or alkoxycarbonyl group or a substituted or unsubstituted alkyl, cycloalkyl, phenyl, amino, acyl, carbamoyl, alkanesulfonyl, iminomethyl or amidino group and R.sup.7 is a hydrogen atom or a substituted or unsubstituted alkyl, alkoxy, phenyl, cycloalkyl or heterocyclic group, or R.sup.6 and R.sup.7, when taken together with the nitrogen atom to which the two are bonded, form a 3- to 7-membered, substituted or unsubstituted heterocyclic group; R.sup.5 is a substituted or unsubstituted phenyl, thienyl, furyl or pyridyl group; Z is an oxygen or sulfur atom or an imino group; and the broken line means a single or double bond.

2. The method of claim 1, wherein R.sup.1 is an unsubstituted or halogen-substituted lower alkyl, lower alkenyl or aryl group; R.sup.2 is a hydrogen atom or an alkyl or acyl group; R.sup.3 is a hydrogen or

halogen atom or a cyano, azido, carboxyl, hydroxyl, formyl or alkoxycarbonyl group or a substituted or unsubstituted alkyl, alkoxy, phenoxy, cycloalkyl, carbamoyl, amino or phenyl group; R.sup.4 is a hydrogen or halogen atom, a nitro, cyano, carboxyl, acyl, hydroxyl or alkoxycarbonyl group, or a substituted or unsubstituted alkyl, alkoxy, alkylthio, phenylthio, lower alkynyl, lower alkenyl, sulfamoyl, lower alkanesulfinyl, lower alkanesulfonyl, amidino, phenyl or heterocyclic group or a group of the formula ##STR6## where R.sup.6 is a hydrogen atom, a hydroxyl, cyano or alkoxycarbonyl group or a substituted or unsubstituted alkyl, cycloalkyl, phenyl, amino, acyl, carbamoyl, alkanesulfonyl, iminomethyl or amidino group and R.sup.7 is a hydrogen atom or a substituted or unsubstituted alkyl, alkoxy, phenyl, cycloalkyl or heterocyclic group, or R.sup.6 and R.sup.7, when taken together with the nitrogen atom to which the two are bonded, form a 3- to 7-membered substituted or unsubstituted heterocyclic group; R.sup.5 is a substituted or unsubstituted phenyl, thienyl, furyl or pyridyl group; Z is an oxygen or sulfur atom or an imino group; and the broken line means a single or double bond.

3. The method of claim 1, wherein R.sup.1 is an unsubstituted or halogen-substituted alkyl group; R.sup.2 is a hydrogen atom; R.sup.3 is a hydrogen atom or a substituted or unsubstituted alkyl group; R.sup.4 is a hydrogen atom, a carboxyl group or a substituted or unsubstituted alkylthio or acylamino group or a carbamoyl group; R.sup.5 is a substituted or unsubstituted phenyl group; Z is an oxygen atom or an imino group and the broken line means a double bond.

4. The method of claim 1, wherein R.sup.1 is an unsubstituted or halogen-substituted lower alkyl group; R.sup.2 is a hydrogen atom; R.sup.3 is a hydrogen atom or a substituted or unsubstituted alkyl group; R.sup.4 is a hydrogen atom, a carboxyl group or a substituted or unsubstituted alkylthio or acylamino group or a carbamoyl group; R.sup.5 is a substituted or unsubstituted phenyl group; Z is an oxygen atom or an imino group and the broken line means a double bond.

5. The method of claim 3, wherein R.sup.3 is a hydrogen atom; R.sup.4 is a substituted or unsubstituted acylamino group; and Z is an oxygen atom.

6. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

7. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

8. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 6-(2,4-difluorophenoxy)-7-methylsulfonylamino-4H-1-benzopyran-4-one.

9. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-carbamoyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

10. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-carbamoyl-2-methyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one

11. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-(N-formyl-N-methyl)amino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

12. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-carboxy-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

13. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-methylthio-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

14. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 6-(2,4-difluorophenylamino)-3-formylamino-7-methylsulfonylamino-4H-1-benzopyran-4-one.

15. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 3-carbamoyl-6-(2,4-difluorophenylamino)-7-methylsulfonylamino-4H-1-benzopyran-4-1-one.

16. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 2-methyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

17. The method of claim 1, wherein said 4H-1-benzopyran-4-one compound is 6-(2-fluorophenylamino)-3-formylamino-7-methylsulfonylamino-4H-1-benzopyran-4-one.

18. The method of claim 1, wherein said autoimmune disease is chronic rheumatoid arthritis, systemic lupus erythematosus, **scleroderma** , mixed connective tissue disease, polyarteritis nodosa, polymyositis/dermatomyositis, Sjogren's syndrome, Bechet's disease, multiple sclerosis, autoimmune diabetes, Hashimoto's disease, psoriasis, primary myxedema, pernicious anemia, myasthenia gravis, ulcerative colitis, chronic active hepatitis, autoimmune hemolytic anemia, or idiopathic thrombocytopenic purpura.

19. A method of inhibiting cell adhesion, comprising: administering to a patient in need of same a cell adhesion inhibiting amount of a 4H-1-benzopyran-4-one compound represented by the following formula or a salt thereof: ##STR7## wherein R.sup.1 is an unsubstituted or halogen-substituted alkyl, alkenyl or aryl group; R.sup.2 is a hydrogen atom or an alkyl or acyl group; R.sup.3 is a hydrogen or halogen atom or a cyano, azido, carboxyl, hydroxyl, formyl or alkoxycarbonyl group or a substituted or unsubstituted alkyl, alkoxy, phenoxy, cycloalkyl, carbamoyl, amino or phenyl group; R.sup.4 is a hydrogen or halogen atom, a nitro, cyano, carboxyl, acyl, hydroxyl or alkoxycarbonyl group, or a substituted or unsubstituted alkyl, alkoxy, alkylthio, phenylthio, alkynyl, alkenyl, sulfamoyl, alkanesulfinyl, alkanesulfonyl, amidino, phenyl or heterocyclic group or a group of the formula ##STR8## where R.sup.6 is a hydrogen atom, a hydroxyl, cyano or alkoxycarbonyl group or a substituted or unsubstituted alkyl, cycloalkyl, phenyl, amino, acyl, carbamoyl, alkanesulfonyl, iminomethyl or amidino group and R.sup.7 is a hydrogen atom or a substituted or unsubstituted alkyl, alkoxy, phenyl, cycloalkyl or heterocyclic group, or R.sup.6 and R.sup.7, when taken together with the nitrogen atom to which the two are bonded, form a 3- to 7-membered, substituted or unsubstituted heterocyclic group; R.sup.5 is a substituted or unsubstituted phenyl, thienyl, furyl or pyridyl group; Z is an oxygen or sulfur atom or an imino group; and the broken line means a single or double bond.

20. The method of claim 19, wherein R.sup.1 is an unsubstituted or halogen-substituted lower alkyl, lower alkenyl or aryl group; R.sup.2 is a hydrogen atom or an alkyl or acyl group; R.sup.3 is a hydrogen or halogen atom or a cyano, azido, carboxyl, hydroxyl, formyl or alkoxycarbonyl group or a substituted or unsubstituted alkyl, alkoxy, phenoxy, cycloalkyl, carbamoyl, amino or phenyl group; R.sup.4 is a hydrogen or halogen atom, a nitro, cyano, carboxyl, acyl, hydroxyl or alkoxycarbonyl group, or a substituted or unsubstituted alkyl, alkoxy, alkylthio, phenylthio, lower alkynyl, lower alkenyl, sulfamoyl, lower alkanesulfinyl, lower alkanesulfonyl, amidino, phenyl or heterocyclic group or a group of the formula ##STR9## where R.sup.6 is a hydrogen atom, a hydroxyl, cyano or alkoxycarbonyl group or a substituted or unsubstituted alkyl, cycloalkyl, phenyl, amino, acyl, carbamoyl, alkanesulfonyl, iminomethyl or amidino group and R.sup.7 is a hydrogen atom or a substituted or unsubstituted alkyl, alkoxy, phenyl, cycloalkyl or heterocyclic group, or R.sup.6 and R.sup.7, when taken together with

the nitrogen atom to which the two are bonded, form a 3- to 7-membered substituted or unsubstituted heterocyclic group; R.sup.5 is a substituted or unsubstituted phenyl, thienyl, furyl or pyridyl group; Z is an oxygen or sulfur atom or an imino group; and the broken line means a single or double bond.

21. The method of claim 19, wherein R.sup.1 is an unsubstituted or halogen-substituted alkyl group; R.sup.2 is a hydrogen atom; R.sup.3 is a hydrogen atom or a substituted or unsubstituted alkyl group; R.sup.4 is a hydrogen atom, a carboxyl group or a substituted or unsubstituted alkylthio or acylamino group or a carbamoyl group; R.sup.5 is a substituted or unsubstituted phenyl group; Z is an oxygen atom or an imino group and the broken line means a double bond.

22. The method of claim 19, wherein R.sup.1 is an unsubstituted or halogen-substituted lower alkyl group; R.sup.2 is a hydrogen atom; R.sub.3 is a hydrogen atom or a substituted or unsubstituted alkyl group; R.sup.4 is a hydrogen atom, a carboxyl group or a substituted or unsubstituted alkylthio or acylamino group or a carbamoyl group; R.sup.5 is a substituted or unsubstituted phenyl group; Z is an oxygen atom or an imino group and the broken line means a double bond.

23. The method of claim 21, wherein R.sup.3 is a hydrogen atom; R.sup.4 is a substituted or unsubstituted acylamino group; and Z is an oxygen atom.

24. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

25. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

26. The method of claim-19, wherein said 4H-1-benzopyran-4-one compound is 6-(2,4-difluorophenoxy)-7-methylsulfonylamino-4H-1-benzopyran-4-one.

27. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-carbamoyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

28. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-carbamoyl-2-methyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

29. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-(N-formyl-N-methyl)amino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

30. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-carboxy-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

31. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-methylthio-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

32. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 6-(2,4-difluorophenylamino)-3-formylamino-7-methylsulfonylamino-4H-1-benzopyran-4-one.

33. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 3-carbomoyl-6-(2,4-difluorophenylamino)-7-methylsulfonylamino-4H-1-benzopyran-4-one.

34. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound is 2-methyl-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one.

35. The method of claim 19, wherein said 4H-1-benzopyran-4-one compound

is 6-(2-fluorophenylamino)-3-formylamino-7-methylsulfonylamino-4H-1-benzopyran-4-one.

L6 ANSWER 8 OF 16 USPATFULL
ACCESSION NUMBER: 1998:115564 USPATFULL
TITLE: Monoclonal antibodies to nucleolar protein
INVENTOR(S): Aris, John, New York, NY, United States
Blobel, Gunter, New York, NY, United States
PATENT ASSIGNEE(S): The Rockefeller University, New York, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5811247		19980922
APPLICATION INFO.:	US 2037173		19940228 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. 995340, filed on 22 Dec 1992, now abandoned which is a continuation of Ser. No. 376435, filed on 7 Jul 1989, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Spiegel, Carol A.
LEGAL REPRESENTATIVE: Felfe & Lynch
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 5
NUMBER OF DRAWINGS: 30 Drawing Figure(s); 14 Drawing Page(s)
LINE COUNT: 1048
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a hybridoma cell line which produces a monoclonal antibody which cross reacts with both yeast and human fibrillarin. Diagnostic kits are also described. These are useful in diagnosing diseases such as **scleroderma**.

CLM What is claimed is:

1. Monoclonal antibody which specifically binds to yeast fibrillarin.

2. The monoclonal antibody of claim 1, wherein said monoclonal antibody also binds to human fibrillarin and is designated HB 11956.

3. Hybridoma cell line which produced a monoclonal antibody which specifically binds to yeast fibrillarin.

4. The hybridoma cell line of claim 3, wherein said monoclonal antibody binds to both yeast fibrillarin and human fibrillarin and is designated HB 11956.

5. Test kit useful in diagnosing **scleroderma**, comprising: (i) a positive control reagent which comprises monoclonal antibody designated HB11956 which specifically binds to both human fibrillarin and to yeast fibrillarin; and (ii) a receptor which specifically binds to said monoclonal antibody which specifically binds to said human fibrillarin.

6. The test kit of claim 5, further comprising a solid phase which has immobilized thereon a sample of said human fibrillarin.

7. The test kit of claim 5, wherein said receptor is a second antibody which specifically binds to said monoclonal antibody.

8. The test kit of claim 7, wherein said second antibody is labelled fluorescently, enzymatically or chromophorically.

L6 ANSWER 9 OF 16 USPATFULL
ACCESSION NUMBER: 1998:61386 USPATFULL

TITLE: Diagnosis of scleroderma and related diseases
INVENTOR(S): Nelson, J. Lee, Seattle, WA, United States
PATENT ASSIGNEE(S): Fred Hutchinson Cancer Research Center, Seattle, WA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5759766		19980602
APPLICATION INFO.:	US 1996-683888		19960719 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-1315P	19950721 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Myers, Carla J.
LEGAL REPRESENTATIVE: Townsend and Townsend and Crew LLP
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
LINE COUNT: 681
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Allogeneic cells are removed from an individual predisposed to or suffering from **scleroderma** or related diseases, thereby treating the disease and inhibiting or preventing its recurrence. Allogeneic cells are identified in the individual and treatment tailored to remove such cells, in vivo or ex vivo, from the individual by cell separation or cytotoxic agents.

CLM What is claimed is:

1. A method for diagnosing a predisposition to developing or suffering from disease, other than graft-versus host disease, associated with allogeneic cellular microchimerism in an individual, comprising: removing a sample of cells from said individual; and determining whether said sample contains allogeneic cells, the presence of which thereby establishing said predisposition to developing or suffering from said disease.

2. The method of claim 1, further comprising, before said determining step, the step of enriching the sample of cells removed from the individual for stem cells or fetal cells.

3. The method of claim 1, wherein the enrichment for stem cells or fetal cells is performed by immunoaffinity chromatography.

4. The method of claim 1, wherein the individual is female and determining whether said sample contains allogeneic cells is by probing for polynucleotide sequences specific for the Y gene in the sample.

5. The method of claim 4, wherein the probing for polynucleotide sequences specific for the Y gene is performed by polymerase chain reaction.

6. The method of claim 1, wherein determining whether said sample contains allogeneic cells is by HLA typing.

7. The method of claim 1, wherein the sample of cells is obtained from the blood of said individual.

8. The method of claim 1, wherein the sample of cells is obtained from the lung of said individual.

9. The method of claim 1, wherein the disease associated with the allogeneic cellular microchimerism is **scleroderma**.

L6 ANSWER 10 OF 16 USPATFULL
ACCESSION NUMBER: 97:120459 USPATFULL
TITLE: Diagnostic method, test kit, drug and therapeutic treatment for autoimmune diseases
INVENTOR(S): Salonen, Eeva-Marjatta, Tunturikatu 15 B 46, FIN-00100 Helsinki, Finland

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5700641		19971223
APPLICATION INFO.:	US 1995-396238		19950301 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Saunders, David
LEGAL REPRESENTATIVE: Browdy and Neimark
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 1
LINE COUNT: 728
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Synthesized telomeric sequences bind to and can be used for detecting anti-DNA antibodies in serum. Autoimmune diseases such as Lupus Erythematosus, Rheumatoid Arthritis, and **Scleroderma**, can be detected by detecting an elevated level of anti-DNA antibodies using telomeric sequences. Test kits for such detection are provided including immobilized telomeric sequences capable of binding anti-DNA antibodies. Pharmaceutical compositions for inhibiting or reducing the activity of anti-DNA antibodies contain an effective amount of telomeric sequences effective in inhibiting the antibodies specific to the patient treated.

CLM What is claimed is:

1. A method for detecting anti-DNA-antibodies in a sample by contacting said sample with a telomeric sequence of DNA selected from the group consisting of a single stranded telomere, a complementary strand thereto, a double stranded teleomere, and a part or a repeat or a combination of any of the foregoing, to provide binding of said antibodies to said telomeric sequence of DNA, and detecting said binding of said antibodies to said telomeric sequence.

2. The method according to claim 1, wherein the telomeric sequence is selected from the group consisting of the human telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double stranded human telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.

3. The method according to claim 2, wherein said repeat of said telomere strand is selected from the group consisting of 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3' (SEQ ID NO:1) 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3' (SEQ ID NO:2), and a duplex thereof.

4. The method according to claim 1 said sample is mammalian serum.

5. The method according 4, wherein the serum is human serum.

6. A diagnostic method for detecting autoimmune diseases in mammals by contacting a serum sample from said mammal with telomeric sequence of DNA which is specific to said mammal and which is selected from the group consisting of a single stranded telomere, a complementary strand thereto, a double stranded telomere, a part or a repeat or a combination of any of the foregoing, to detect an elevated level of autoantibodies present in said mammals above a normal level of autoantibodies.

7. The diagnostic method of claim 6 wherein said autoimmune disease is selected from the group consisting of Lupus Erythematosus, Rheumatoid Arthritis and **Scleroderma**.

8. The diagnostic method of claim 7 wherein said autoimmune disease is Systemic Lupus Erythematosus (SLE).

9. The diagnostic method according to claim 6, wherein said telomeric sequence is selected from the group consisting of the human telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double stranded human telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.

10. The diagnostic method according to claim 9, wherein said repeat of said telomere strand is selected from the group consisting of 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3' (SEQ ID NO:1) 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3' (SEQ ID NO:2), and a duplex thereof.

11. The diagnostic method of claim 6, wherein the immunoglobulin (Ig) class of said autoantibodies is determined with the aid of anti-Ig-antibodies.

12. The diagnostic method according to claim 9, wherein the existence of SLE in a human patient is detected by contacting a sample of said patient's serum with an immobilized human telomeric sequence to bind autoantibodies present in said serum to said immobilized telomeric sequence and detecting an elevated level of bound IgG class autoantibodies among said bound autoantibodies.

13. The diagnostic method according to claim 9, wherein the existence of SLE in a human patient is detected by contacting a sample of said patient's serum with an immobilized human telomeric sequence to bind autoantibodies present in said serum to said immobilized telomeric sequence and detecting an elevated level of bound IgM class autoantibodies among said bound autoantibodies.

14. A test kit for the diagnosis of autoimmune disease in mammals, said test kit including a telomeric sequence of DNA selected from the group consisting of a single stranded telomere, a complementary strand thereto, a double stranded telomere, a part or a repeat or a combination of any of the foregoing, capable of binding autoantibodies present in said serum, wherein said telomeric sequence is immobilized on a solid support and a label capable of indicating the binding of said autoantibody to said telomeric sequence.

15. The test kit according to claim 14 wherein said telomeric sequence is selected from the group consisting of the human telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double stranded human telomere, a functional part of any of said strands, a repeat of any of said strands and a combination of any of the foregoing.

16. The test kit according to claim 15, wherein said repeat of said telomere strand is selected from the group consisting of 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3' (SEQ ID NO:1) 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3' (SEQ ID NO:2), and a duplex thereof.

17. The test kit according to claim 14, wherein said kit further comprises a labeled telomeric sequence.

18. The test kit according to claim 14 wherein said label is selected from the group consisting of a radio label, an enzyme label, a fluorochrome label, a dye, a sol, biotin, and a luminescent label.

19. The test kit according to claim 14, wherein the label is provided as one or more labeled anti-Ig-antibodies selected from the group consisting of anti-IgG, anti-IgM, anti-IgA, anti-IgD, and anti-IgE.

20. The test kit according to claim 19 wherein said anti-Ig antibody is

anti-IgG or anti-IgM.

21. A test kit for the diagnosis of autoimmune disease in mammals, said test kit including a telomeric sequence of DNA selected from the group consisting of a dingle stranded telomere, a complementary strand thereto, a double stranded telomere, a part or a repeat or a combination of any of the foregoing, capable of binding autoantibodies present in said serum; and an amount of one or more labelled anti-Ig-antibodies selected from the group consisting of anti-IgG, anti-IgM, anti-IgA, anti-IgD and anti-IgE.

22. The test kit according to claim 21, wherein said telomeric sequence is immobilized on a solid support.

23. The test kit according to claim 21 wherein said telomeric sequence is selected from the group consisting of the human telomere strand 5'-TTAGGG-3', the complementary strand thereto, a double stranded human telomere, a functional part of any of said strands, a repeat of any of said strands, and a combination of any of the foregoing.

24. The test kit according to claim 23 wherein said repeat of said telomere strand is selected from the group consisting of 5'-TTAGGG TTAGGG TTAGGG TTAGGG TTAGGG-3' SEQ ID NO:1 5'-CCCTAA CCCTAA CCCTAA CCCTAA CCCTAA-3' SEQ ID NO:2 and a duplex thereof.

25. The test kit according to claim 21 wherein said label is selected from the group consisting of a radio label, an enzyme label, a fluorochromic label, a dye, a sol, biotin, and a luminescent label.

26. The test kit according to claim 21 wherein said anti-Ig-antibody is anti-IgG or anti-IgM.

L6 ANSWER 11 OF 16 USPATFULL

ACCESSION NUMBER:	96:63111 USPATFULL
TITLE:	S-nitroso derivatives of hydrazinoacetic acids, 1-[(acylthio and (mercapto)-1-oxoalkyl]-1,2,34-Tetrahydroquinoline-2-carboxylic acids and alanyl prolines and isoquinolines
INVENTOR(S):	Loscalzo, Joseph, Dedham, MA, United States Cooke, John, Needham Heights, MA, United States
PATENT ASSIGNEE(S):	Brigham & Women's Hospital, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5536723		19960716
APPLICATION INFO.:	US 1994-319414		19941006 (8)

RELATED APPLN. INFO.:	Division of Ser. No. US 1993-13404, filed on 4 Feb 1993, now patented, Pat. No. US 5356890 which is a division of Ser. No. US 1991-715588, filed on 14 Jun 1991, now patented, Pat. No. US 5187183 which is a division of Ser. No. US 1989-328397, filed on 24 Mar 1989, now patented, Pat. No. US 5025001 which is a continuation-in-part of Ser. No. US 1988-206763, filed on 15 Jun 1988, now patented, Pat. No. US 5002964
DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Higel, Floyd D.
LEGAL REPRESENTATIVE:	Herron, Charles J., Olstein, Elliot M.
NUMBER OF CLAIMS:	36
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	11 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT:	1463

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to novel S-nitroso derivatives of ACE inhibitors and to pharmaceutical compositions comprising the S-nitrosothiol derivatives of the invention together with a pharmaceutically acceptable carrier.

The invention also relates to methods for treating various pathophysiological conditions including acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, vascular thrombosis, Raynauds syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, and renal artery stenosis, and to methods of inhibiting ACE and effecting vasodilation comprising administering the S-nitrosothiol derivatives of the ACE inhibitors of the invention to an animal.

CLM What is claimed is:

1. An S-nitrosothiol compound having the formula: ZCHRCON(--N.dbd.CR.sub.4 R.sub.5)CHR.sub.6 (CH.sub.2).sub.n COY in which Z is ON--SCH.sub.2 --; R is hydrogen or C.sub.1 to C.sub.10 alkyl; R.sub.4 and R.sub.5 are each independently selected from hydrogen, phenyl, naphthyl; a heterocyclic group selected from thiophenyl, furyl, pyrrolyl, imidazolyl, oxazolyl, thiazolyl, pyrimidinyl and pyridinyl; cycloalkyl containing 3 to 7 carbon atoms; or C.sub.1 -C.sub.10 alkyl which is unsubstituted or substituted by phenyl, naphthyl or a heterocyclic group selected from thiophenyl, furyl, pyrrolyl, imidazolyl, oxazolyl, thiazolyl, pyrimidinyl and pyridinyl, which pyridinyl is unsubstituted or is fused to a benzene ring; when R.sub.4 or R.sub.5 are phenyl, naphthyl or a heterocyclic group selected from thiophenyl, furyl, pyrrolyl, imidazolyl, oxazolyl, thiazolyl, pyrimidinyl, and pyridyl, said phenyl, napthyl or heterocyclic group is unsubstituted or substituted by one or more C.sub.1 -C.sub.10 alkyl, C.sub.1 -C.sub.10 alkoxy, halogen, C.sub.7 -C.sub.12 phenylalkyl, C.sub.7 -C.sub.12 phenylalkoxy, phenyl, hydroxy, carbonyl, C.sub.1 -C.sub.10 fluoroalkyl, cyano, nitro, phenylsulphonamido, C.sub.2 -C.sub.20 dialkyl-amino-alkoxy, C.sub.1 -C.sub.10 alkylthio, or C.sub.2 -C.sub.20 dialkyl-amino, or R.sub.4 and R.sub.5 together form a --(CH.sub.2).sub.4 --, --(CH.sub.2).sub.5 --, ##STR60## R.sub.6 is hydrogen or C.sub.1 -C.sub.10 alkyl; q is 2 or 3; n is 0 or 1; Y is hydroxy or --NHSO.sub.2 R.sub.9 ; and R.sub.9 is C.sub.1 -C.sub.10 alkyl, or pharmaceutically acceptable salts, esters or amides thereof.

2. A pharmaceutical composition comprising the S-nitrosothiol compound of claim 1 and a pharmaceutically acceptable carrier.

3. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical compsoition of claim 1 to an animal.

4. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 2 to an animal.

5. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 2 to an animal.

6. A method of effecting vasodilation comprising administering the pharmaceutical composition of claim 1 to an animal.

7. An S-nitrosothiol compound having the formula: ##STR61## R.sub.2 is hydrogen, methoxy, or methyl; R.sub.3 is hydrogen, methoxy, methyl,

chloro, or hydroxy; R.sub.4 is hydrogen, methyl or acetylthiomethyl; m is 0 or 1 or the sodium or dicyclohexylamine salts thereof.

8. A pharmaceutical composition comprising the S-nitrosothiol compound of claim 7 and a pharmaceutically acceptable carrier.

9. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical compsoition of claim 8 to an animal.

10. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 8 to an animal.

11. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 8 to an animal.

12. A method of effecting vasodilation comprising administering the pharmaceutical composition of claim 8 to an animal.

13. An S-nitrosothiol compound having the formula: ##STR62## wherein A and A' are independently hydroxy, lower alkoxy or benzyloxy; R.sub.1 is hydrogen or lower alkyl; R.sub.2, R.sub.3, R.sub.4 and R.sub.5 are independently hydrogen, lower alkyl or amino-lower alkyl M and M' are connected together to form an alkylene bridge of from 2 to 5 carbon atoms or together with the N and C to which they are connected form a 1,2,3,4-tetrahydro-isoquinoline ring; and Z is ON--S--(CH.sub.2).sub.2 --, ON--S--(CH.sub.2).sub.3 --, ON'S--(CH.sub.2).sub.4 --, ON--S--(CH.sub.2).sub.5 -- or their pharmaceutically acceptable salts.

14. A pharmaceutical composition comprising the S-nitrosothiol compound of claim 13 and a pharmaceutically acceptable carrier.

15. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris or vascular thrombosis, comprising administering the pharmaceutical composition of claim 14 to an animal in need thereof.

16. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 14 to an animal in need thereof.

17. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 14 to an animal in need thereof.

18. A method of effecting vasodilation comprising administering the pharmaceutical 4composition of claim 14 to an animal in need thereof.

19. An S-nitrosothiol compound having the formula: ##STR63## wherein A is a group having the formula --Q.sub.k --(NQ.sup.1).sub.m --(CH.sub.2).sub.n --, where k and m are each independently selected from 0 and 1, provided that m can only be 0 when k is 0, n is from 1 to 6, Q.sup.1 is hydrogen or C.sub.1-4 alkyl, Q is --CO--, --CH.sub.2 --, --CH.sub.2 CO-- or --OCH.sub.2 CO--, and any of the --(CH.sub.2).sub.n -- groups are each independently unsubstituted or substituted by one or two C.sub.1-4 alkyl groups; B is a group of formula --R.sup.1 --CQ.sup.2 (OH)--C(Q.sup.3)(Q.sup.4)--NQ.sup.5 --R.sup.2, where R.sup.1 is a bond

or --OC(Q.sup.6)(Q.sup.7)--, R.sup.2 is hydrogen or C.sub.1-6 alkyl, and Q.sup.2 -Q.sup.7 are each independently hydrogen, C.sub.1-4 alkyl, or a group of formula --(C(Q.sup.8)(Q.sup.9)).sub.x Ph, in which x is 1 or 2, Ph is a phenyl group which is unsubstituted or substituted by one or more groups independently selected from hydroxy, C.sub.1-4 alkyl, and C.sub.1-4 alkoxy, and Q.sup.8 and Q.sup.9 are independently hydrogen or C.sub.1-4 alkyl; Z is carboxyl; D is hydrogen or a saturated or unsaturated C.sub.1-6 aliphatic substituent which is unsubstituted or substituted by an amino group; G.sup.1, G.sup.2 and G.sup.3 are each independently selected from hydrogen and C.sub.1-4 alkyl; (X) is an aromatic ring system having the formula: ##STR64## where .beta. is the point of attachment of B, .alpha. is the point of attachment of A, M is a pyrrole, pyridine, or benzene ring, y and z are independent integers of from 0 to 3, and R.sup.3 and R.sup.4 are independently selected from halo, C.sub.1-4 alkyl and hydroxy; and (Y) is a nitrogen-containing ring system selected from the group consisting of pyrrolyl, imiadazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperidinyl, piperizinyl, 1H-indolyl, 3H-indolyl, oxazolidinyl, -quinolyl, tetrahydroquinolyl and morpholino; or physiologically acceptable salts thereof; with the proviso that: when, in the definition of A, k and m are both 0 or both 1 and Q is a --CO-- group, and, in the definition of B, R.sup.1 is a group of formula --OC(Q.sup.6)(Q.sup.7)-- as hereinbefore defined and R.sup.2 is a C.sub.1-6 alkyl group; D is hydrogen or a C.sub.1-6 alkyl group; (X) is a benzene ring or a naphthyl or indolyl ring system, any of which is unsubstituted or is substituted in any position by one or more substituents independently selected from C.sub.1-4 alkyl, C.sub.1-4 alkoxy, halo, nitro, amino, carboxyl, C.sub.1-4 alkoxycarbonyl and hydroxy, then (Y) is not a pyrrolidinyl, oxazolidinyl or thiazolidinyl ring, or an indolinyl, quinolinyl or tetrahydroquinolinyl ring system, with the further proviso that at least one of the alkyl substitutents is further substituted with an --SNO group.

20. A pharmaceutical composition comprising the S-nitrosothiol compound of claim 19 and a pharmaceutically acceptable carrier.

21. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical composition of claim 20 to an animal.

22. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 20 to an animal.

23. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 20 to an animal.

24. A method of effecting vasodilation comprising administering the pharmaceutical composition of claim 20 to an animal.

25. An S-nitrosothiol compound having the formula: ##STR65## wherein Q and Q.sup.1 -Q.sup.8 are each independently hydrogen or C.sub.1-4 alkyl; A is a group of formula --(CO).sub.k --(NQ.sup.9).sub.m --(CH.sub.2).sub.n -- where k and m are either both 0 or both 1, n is from 1 to 6, Q.sup.9 is selected from hydrogen and C.sub.1-4 alkyl and any of the --(CH.sub.2).sub.n -- groups independently are unsubstituted or substituted by one or two C.sub.1-4 alkyl groups; B is a C.sub.1-6 alkyl group; E and Z are each carboxy; D is hydrogen or a C.sub.1-6 alkyl group which is unsubstituted or substituted by an amino group; (X)

is a benzene ring or a naphthyl or indolyl ring system any of which is unsubstituted or is substituted in any position by one or more substitutents independently selected from C.sub.1-4 alkyl, C.sub.1-4 alkoxy, halo, nitro, amino, carboxy, C.sub.1-4 alkoxycarbonyl and hydroxy; and --N Y is a pyrrolidinyl, oxazolidinyl or thiazolidinyl ring, or an indolinyl, quinolinyl or tetrahydroquinolinyl ring system; wherein at least one of the alkyl groups is substituted by an --SNO group.

26. A pharmaceutical composition comprising the S-nitrosothiol compound of claim 25 and a pharmaceutically acceptable carrier.

27. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical compsoition of claim 26 to an animal.

28. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 26 to an animal.

29. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 26 to an animal.

30. A method of effecting vasodilation comprising administering the pharmaceutical composition of claim 26 to an animal.

31. An S-nitrosothiol compound having a formula selected from the group consisting of: ##STR66## where R.sup.1 and R.sup.7 are the same or different and are hydroxy, alkoxy with up to 10 cabon atoms, aryloxy with 6 to 12 carbon atoms or araloxy with 7 to 14 carbon atoms; R.sup.2 is hydrogen or alkyl with up to 4 carbon atoms; R.sup.3 is hydrogen or is --(CH.sub.2).sub.m --R.sup.14 where m is an integer from 1 to 6; and R.sup.14 is hydrogen, cycloalkyl with 3 to 8 carbon atoms, hydroxy, carboxy, alkoxycarboxyl with up to 4 carbon atoms in the alkyl group, one 5- to 7- member heterocyclic ring with oxygen, sulfur or nitrogen as heteroatoms, aryl with 6 to 12 carbon atoms, whereby these aryl radicals are unsubstituted or substituted by halogen, cyano, nitro, alkyl or alkoxyl with up to 4 carbon atoms; or R.sup.2 and R.sup.3 are joined through a group having the formula: ##STR67## R.sup.4 is hydrogen or --(CH.sub.2).sub.n --R.sup.15, wherein n is defined as previously for m and within a compound n and m are the same or different, and R.sup.15 is defined as previously for R.sup.14 ; R.sup.5 is hydrogen or alkyl with up to 8 carbon atoms and is unsubstituted or substituted by a group having the formula: ##STR68## or is a cycloaklyl group with 3 to 8 carbon atoms and is unannelated or annelated with a phenyl ring, or is a group NR.sup.16 R.sup.17 where R.sup.16, R.sup.17 are the same or different and are hydrogen, alkyl with up to 6 carbon atoms, aryl with 6 to 12 carbon atoms, aralkyl with 7 to 14 carbon atoms or acyl with 2 to 7 carbon atoms; or R.sup.4 and R.sup.5 are joined through a group having the formula: ##STR69## where p is an integer from 1 to 4; A is a direct binding of oxygen, sulfur, NH, N-alkyl with up to 6 carbon atoms; N-phenyl, --CHCOOH or CHCOO-alkyl with up to 6 carbon atoms in the alkyl chain; R.sup.6 is hydrogen or R.sup.6 and R.sup.5 joined through a group according to the formula: ##STR70## wherein X is methylene or a carbonyl group; Y is sulfur, NH, N-alkyl with up to 6 carbon atoms in the alkyl group, N-phenyl, carbonyl group or --CHR.sup.20 group; where R.sup.20 is hydroxy, mercapto, phenyloxy, phenylthio, alkoxy or alkythio with up 6 carbon atoms in the alkyl group; W and Z are the same or different direct binding of methylene or ethylene group; q and r are each independently integers from 1 to 4; R.sup.16 and R.sup.19 are the same

or different and are hydrogen, alkyl or alkoxyl with up to 6 carbon atoms or either of them has the formula ##STR71## with R groups as defined for Formula I ##STR72## where s is 0 or an integer from 1 to 3; B is methylene, ethylene or a vinyl group; and R.sup.21 and R.sup.22 are the same or different and are hydrogen or alkyl with up to 4 carbon atoms or an acyl group with 1 to 4 carbon atoms; R.sup.8 is hydrogen or alkyl group with up to 6 carbon atoms; R.sup.9 is hydrogen or a radical having the formula ##STR73## where t and u are each independently 0 or an integer from 1 to 4; R.sup.23 is hydrogen, alkyl, alkylthio or alkylcarbonyl with up to 6 carbon atoms in alkyl chain, benzoyl or si a radical from the following group ##STR74## R.sup.24 is hydrogen, alkyl or alkylcarbonyl with up to 8 carbon atoms in the alkyl chain, carboxy, alkoxycarbonyl with up to 6 carbon atoms or is a radical from the following group ##STR75## and R.sup.25 is hydrogen or a radical from the group ##STR76## R.sup.10 is hydrogen or an alkyl with up to 6 carbon atoms; R.sup.11 is hydrogen or an alkyl group with up to 8 carbon atoms which is unsubstituted or is substituted by a 5- to 7- membered heterocyclic ring with oxygen, sulfur or nitrogen as heteroatoms, or is a cycloalkyl with 3 to 7 carbon atoms which can be substituted with phenyl or alkyl with up to 4 carbon atoms or R.sup.10 and R.sup.11 joined through a group having the formula ##STR77## where v and w are each independently 0 or an integer from 1 to 4; D may be direct binding of oxygen, sulfur, NH or ortho-phenyl group; R.sup.12 is hydrogen or alkyl with up to 6 carbon atoms which is unsubstituted or is substituted with an indole group or R.sup.11 and R.sup.12 joined through a group having the formula ##STR78## where x and y are each independently 0 or an integer from 1 to 4; R.sup.26 is hydrogen; R.sup.27 is hydrogen, alkyl, with up to 6 carbon atoms, cycloalkyl with 3 to 7 carbon atoms, phenylthio, phenylsulfonyl, alkylthio or alkylsulfonyl with up to 4 carbon atoms, or a radical according to the formula ##STR79## or R.sup.26 and R.sup.27 are joined through a group having the formula ##STR80## ;and R.sup.13 is hydroxy or alkoxy with up to 6 carbon atoms and their physiological salts as positive ionotrope producing dihydropiridine bindings (Component B) having formula III ##STR81## wherein R is cycloalkyl with 3 up to 14 carbon atoms, aryl with 6 to 14 carbon atoms or heteroaryl which is unsubstituted or substituted with any combination of the following: halogen, nitro, trifluoromethyl, monofluoraloxy with up to 12 carbon atoms, polyfluoraloxy with up to 12 carbon atoms, hydroxy, amino, alkylamino with 8 carbon atoms, dialkylamino with up to 8 carbon atoms in the alkyl group, aryl with 6 to 12 carbon atoms, heteroaryl, aralkyl with 7 to 14 carbon atoms, araloxy with 7 to 14 carbon atoms or --SOn -aralkyl with 7 to 14 carbon atoms where n=0-2, whereby the substitution of the last mentioned five groups is also replaced by 5 substitutes including: halogen, nitro, azido, hydroxy, trifluoromethyl, trifluoromethoxy, cyano, amino, alkylamino with up to 8 10 carbon atoms, dialkylamino with up to 8 carbon atoms in alkyl group, alkoxy or alkylthio with up to 4 carbon atoms; R.sup.1 is a ;linear, branched or cyclic saturated or unsaturated carbohydrate chain with up to 20 carbon atoms, with up to 5 sulfurs and/or oxygens which chains can be terminated by one of the following: halogen, nitro, hydroxy, cyano, trialkylsilyl with up to 8 carbon atoms per alkyl group, alkoxycarbonyl with up to 4 carbon atoms, amino, alkylamino with up to 4 carbon atoms or dialkylamino with up to 4 carbon atoms in alkyl group; R.sup.2 and R.sup.4 are each independently hydrogen, amino, cyano, formyl or a linear, branched or cyclic saturated or unsaturated carbohydrates with up to 10 carbon atoms, and are unsubstituted or are substituted with hydroxy, carboxy, alkoxycarbonyl with up to 4 carbon atoms or by halogen; R.sup.3 is hydrogen or a linear, branched or cyclic saturated or unsaturated carbohydrates with up to 10 carbon atoms and is unsubstituted or is substituted with halogen, cyano, hydroxy, amino, alkylamino, dialkylamino, with up to 4 carbon atoms in the alkyl group or by 5- to 7-membered saturated or unsaturated heterocyclic ring containing nitrogen and/or oxygen and/or sulfur as heteroatoms and R.sup.5 is hydrogen, cyano or nitro or R.sup.4

and R.sup.5 are connected through the following structures ##STR82## where a is 1 or 2; and wherein at least one of R.sup.1 to R.sup.13 is substituted by --SNO.

32. A pharmaceutical composition comprising the S-nitrosothiol derivative of claim 31 and a pharmaceutically acceptable carrier.

33. A method for treating Raynaud's syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical compsoition of claim 32 to an animal.

34. A method of inhibiting platelet aggregation comprising administering the pharmaceutical composition of claim 32 to an animal.

35. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 32 to an animal.

36. A method of effecting vasodilation comprising administering the pharmaceutical composition of claim 32 to an animal.

L6 ANSWER 12 OF 16 USPATFULL

ACCESSION NUMBER:	96:58202 USPATFULL
TITLE:	Tetracyclines including non-antimicrobial chemically-modified tetracyclines inhibit excessive glycosylation of different types of collagen and other proteins during diabetes
INVENTOR(S):	Golub, Lorne M., Smithtown, NY, United States Ramamurthy, Nungavarum S., Smithtown, NY, United States McNamara, Thomas F., Port Jefferson, NY, United States Ryan, Maria E., Port Jefferson Station, NY, United States
PATENT ASSIGNEE(S):	The Research Foundation of State University of New York, Albany, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5532227		19960702
APPLICATION INFO.:	US 1994-361116		19941221 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1992-977549, filed on 17 Nov 1992, now abandoned		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Rollins, John W.		
LEGAL REPRESENTATIVE:	Hoffmann & Baron		
NUMBER OF CLAIMS:	11		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	9 Drawing Figure(s); 9 Drawing Page(s)		
LINE COUNT:	929		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for treating mammals suffering from excessive extracellular protein glycosylation which is associated with diabetes, **scleroderma** and progeria by administering to the mammal a tetracycline which effectively inhibits excessive protein glycosylation.

CLM What is claimed is:

1. A method of treating a mammal suffering from conditions associated with a pathologically excessive amount of protein glycosylation comprising administering to the mammal an effective amount of a tetracycline sufficient to inhibit said pathologically excessive amount

of protein glycosylation.

2. The method of claim 1, wherein a protein which is subject to said pathologically excessive amount of protein glycosylation is a somatic protein with exposed amino groups.

3. The method of claim 2, wherein said somatic protein with exposed amino groups is selected from the group consisting of collagen, laminin, albumin, lens crystallins and fibrin.

4. The method of claim 2, where in said protein is associated with pathological conditions including diabetes mellitus, **scleroderma** and progeria.

5. The method of claim 3, wherein said protein is collagen and said pathologically excessive amount of collagen glycosylation results in a pathologically excessive amount of collagen crosslinking.

6. The method of claim 5, wherein said pathologically excessive amount of collagen crosslinking is associated with conditions including diabetes mellitus, **scleroderma** and progeria.

7. The method according to claim 1, wherein the tetracycline is a dedimethylaminotetracycline.

8. The method according to claim 7, wherein the dedimethylaminotetracycline is selected from the group consisting of 4-dedimethylaminotetracycline, 4-dedimethylamino-5-oxytetracycline, 4-dedimethylamino-7chlorotetracycline, 4-hydroxy-4-dedimethylaminotetracycline, 5a,6-anhydro-4-hydroxy-4-dedimethylaminotetracycline, 6.alpha.-deoxy-5-hydroxy-4-dedimethylaminotetracycline, 6-demethyl-6-deoxy-4-dedimethylaminotetracycline, 4-dedimethylamino-12a-deoxytetracycline, 12a-deoxy-4-deoxy-4-dedimethylaminotetracycline, 12a, 4a-anhydro-4-dedimethylaminotetracycline, 7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline and 4-dedimethylamino-11-hydroxy-12a-deoxytetracycline.

9. The method according to claim 1, wherein the tetracycline is selected from the group consisting of 6a-benzylthiomethylenetetracycline, tetracyclinonitrile, the mono-N-alkylated amide of tetracycline, 6-fluoro-6-demethyltetracycline, 11a-chlorotetracycline, tetracycline pyrazole, 6.alpha.-deoxy-5-hydroxy-4-dedimethylaminodoxycycline and 12a-deoxytetracycline and its derivatives.

10. The method according to claim 1, wherein the tetracycline is administered in an amount of from about 0.1 mg/kg per day to about 50.0 mg/kg per day.

11. The method according to claim 10, wherein the tetracycline is administered in an amount of from about 0.3 mg/kg per day to about 15.0 mg/kg per day.

L6 ANSWER 13 OF 16 USPATFULL

ACCESSION NUMBER:	96:38936 USPATFULL
TITLE:	Methods and polycyclic aromatic compound containing compositions for treating T-cell-mediated diseases
INVENTOR(S):	Meruelo, Daniel, Scarborough, NY, United States Lavie, Gad, Tenafly, NJ, United States
PATENT ASSIGNEE(S):	New York University, New York, NY, United States (U.S. corporation)

NUMBER	KIND	DATE

PATENT INFORMATION:	US 5514714	19960507
APPLICATION INFO.:	US 1993-39790	19930330 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1991-784952, filed on 1 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-572085, filed on 23 Aug 1990, now abandoned	
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted	
PRIMARY EXAMINER:	Henley, III, Raymond	
LEGAL REPRESENTATIVE:	Browdy and Neimark	
NUMBER OF CLAIMS:	6	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	15 Drawing Figure(s); 9 Drawing Page(s)	
LINE COUNT:	1179	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB T cell-mediated diseases in mammals are treated using compositions comprising a polycyclic aromatic compound, preferably hypericin or pseudohypericin, and related compounds, including isomers, analogs, derivatives, salts, or ion pairs of hypericin or pseudohypericin. The above composition may be administered in combination with an immunosuppressive agent. Pharmaceutical compositions useful for treating a T cell-mediated disease comprise the above polycyclic aromatic compound, alone or in combination with an immunosuppressive agent. The compositions and methods are useful in treating diseases which include multiple sclerosis, myasthenia gravis, **scleroderma**, polymyositis, graft-versus-host disease, graft rejection, Graves disease, Addison's disease, autoimmune uveoretinitis, autoimmune thyroiditis, pemphigus vulgaris and rheumatoid arthritis. Psoriasis and systemic lups erythematosus. Also provided are methods for diminishing the expression of CD4 Molecules on the surface of a T lymphocyte, and for inducing multidrug resistance in a cell, comprising incubating the cell with an effective concentration of a polycyclic aromatic compound.

CLM What is claimed is:

1. A method for treating a T cell-mediated disease in a mammal in need of such treatment, comprising administering to said mammal a T-cell inhibiting effective amount of at least one polycyclic aromatic dione compound or a salt thereof, wherein said disease is selected from the group consisting of graft versus host disease, graft rejection and an autoimmune disease.

2. A method according to claim 1, wherein said compound is selected from the group consisting of hypericin, pseudohypericin, desmethyl hypericin, hypericin diacetate, hypericin hexaacetate, hypericin methyl ester, hypericin propyl ester, isopropyl desmethyl hypericin, butyl ester of hypericic acid, sodium hypericin, potassium hypericin, lithium hypericin, hypericin-lysine, hypericin-glutamine, hypericin-ethylenediamine and hypericin-TRIS.

3. A method according to claim 2, wherein said compound is hypericin or pseudohypericin.

4. A method according to claim 1, wherein said disease is an autoimmune disease selected from the group consisting of multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, **scleroderma**, polymyositis, Graves disease, Addison's disease, psoriasis, autoimmune uveoretinitis, autoimmune thyroidiris, Pemphigus vulgaris and rheumatoid arthritis.

5. A method according to claim 1, wherein said disease is graft-versus-host disease.

6. A method according to claim 1, wherein said disease is graft rejection.

L6 ANSWER 14 OF 16 USPATFULL

ACCESSION NUMBER:	94:91047 USPATFULL
TITLE:	S-nitroso derivatives of ace inhibitors and the use thereof
INVENTOR(S):	Loscalzo, Joseph, Dedham, MA, United States Cooke, John, Needham Heights, MA, United States
PATENT ASSIGNEE(S):	Brigham and Women's Hospital, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5356890		19941018
APPLICATION INFO.:	US 1993-1340		19930204 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1991-715588, filed on 14 Jun 1991, now patented, Pat. No. US 5187183, issued on 16 Feb 1993 which is a division of Ser. No. US 1989-328397, filed on 24 Mar 1989, now patented, Pat. No. US 5025001, issued on 18 Jun 1991 which is a continuation-in-part of Ser. No. US 1988-206763, filed on 15 Jun 1988, now patented, Pat. No. US 5002964, issued on 26 Mar 1991

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Higel, Floyd D.
LEGAL REPRESENTATIVE:	Sterne, Kessler, Goldstein & Fox
NUMBER OF CLAIMS:	15
EXEMPLARY CLAIM:	1,2
NUMBER OF DRAWINGS:	11 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT:	1160

AB The invention relates to novel S-nitroso derivatives of ACE inhibitors and to pharmaceutical compositions comprising the S-nitrosothiol derivatives of the invention together with a pharmaceutically acceptable carrier.

The invention also relates to methods for treating various pathophysiological conditions including acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, vascular thrombosis, Raynauds syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, and renal artery stenosis, and to methods of inhibiting ACE and effecting vasodilation comprising administering the S-nitrosothiol derivatives of the ACE inhibitors of the invention to an animal.

CLM What is claimed is:

1. An S-nitrosothiol derivative having the formula: ##STR60## wherein n is 1-5.

2. An S-nitrosothiol having the following formula: ##STR61## wherein, R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5 or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, aryl, or C.sub.1 -C.sub.4 alkyl substituted by aryl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl, or C.sub.1 -C.sub.4 carboxylic acyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, aryloxy or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.7 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 1 or 3; and n is 0 to 2 .

3. A pharmaceutical composition comprising the S-nitrosothiol derivative of claim 1 or 2, and a pharmaceutically acceptable carrier.

4. The pharmaceutical composition of claim 3, in the form of a liquid, a

suspension, a tablet, a dragee, an injectable solution, or a suppository.

5. The pharmaceutical composition of claim 3, comprising per-unit dose, 1 pmole to 10 mmole of said compound.

6. A method for treating Raynauds syndrome, **scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, renal artery stenosis, acute myocardial infarction, left ventricular dysfunction without overt heart failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, or vascular thrombosis, comprising administering the pharmaceutical composition of claim 3 to an animal.

7. The method of claim 6, wherein said animal is a human.

8. The method of claim 6, wherein said pharmaceutical composition comprises, per-unit dose, 1 pmole to 10 mmole of said compound, administered 1 to 4 times daily.

9. The method of claim 6, wherein said pharmaceutical composition is in the form of an injectable solution which is administered by continuous intravenous infusion.

10. A method of inhibiting platelet aggregation, comprising administering the pharmaceutical composition of claim 6 to an animal.

11. A method of inhibiting the activity of angiotensin converting enzyme comprising administering the pharmaceutical composition of claim 6 to an animal.

12. A method of effecting vasodilation, comprising administering the pharmaceutical composition of claim 6 to an animal.

13. The method of any one of claims 10-12, wherein said animal is a human.

14. The method of any one of claims 10-12, wherein said pharmaceutical composition comprises, per unit dose, 1 pmole to 10 mmole of said compound, administered 1 to 4 times daily.

15. The method of any one of claims 10-12 wherein said pharmaceutical composition is in the form of an injectable solution which is administered by continuous intravenous infusion.

L6 ANSWER 15 OF 16 USPATFULL

ACCESSION NUMBER:	91:24673 USPATFULL
TITLE:	S-nitrosocaptopril compounds and the use thereof
INVENTOR(S):	Loscalzo, Joseph, Dedham, MA, United States
PATENT ASSIGNEE(S):	Brigham & Women's Hospital, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5002964		19910326	
APPLICATION INFO.:	US 1988-206763		19880615	(7)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Brust, Joseph Paul			
LEGAL REPRESENTATIVE:	Sterne, Kessler, Goldstein & Fox			
NUMBER OF CLAIMS:	17			
EXEMPLARY CLAIM:	5			
NUMBER OF DRAWINGS:	5 Drawing Figure(s); 5 Drawing Page(s)			

LINE COUNT: 717
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to novel nitroso compounds having the formula: ##STR1## wherein, R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5 or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, aryl, or C.sub.1 -C.sub.4 alkyl substituted by aryl;

R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl, or C.sub.1 -C.sub.4 acyl;

R.sup.3 is hydrogen, C.sub.1 -C.sub.7 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl;

n is 0 to 2;

A is hydrogen,

lower C.sub.1 -C.sub.7 alkyl,

lower C.sub.2 -C.sub.7 alkylene,

lower C.sub.2 -C.sub.7 alkylene substituted by hydroxy, C.sub.1 -C.sub.4 alkyl, aryl, or

a C.sub.4 -C.sub.7 ring which may be fused to a benzene ring;

B is hyrogen,

lower C.sub.1 -C.sub.7 alkyl,

phenyl,

lower C.sub.1 -C.sub.7 substituted by phenyl, hydroxy, guanidino, amino, imidazoyl, indolyl, mercapto, mercapto substituted by lower C.sub.1 -C.sub.4 alkyl, carbamoyl, or carboxyl, or

lower C.sub.2 -C.sub.7 alkylene.

The invention also relates to compounds having the following formula: ##STR2## wherein R, R.sup.1, R.sup.3 and n are as defined above; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, aryloxy or C.sub.1 -C.sub.7 alkyl; and m is 1 to 3.

The invention also relates to pharmaceutical compositions comprising the nitrosothiol compounds of the invention together with a pharmaceutically acceptable carrier.

The invention also relates to methods for treating acute myocardial infarction, left ventricular dysfunction without overt heat failure, hypertension, pulmonary hypertension, congestive heart failure, angina pectoris, vascular thrombosis, Raynauds syndrome, **Scleroderma**, toxemia of pregnancy, acute renal failure, diabetic nephropathy, and renal artery stenosis, and to methods of inhibiting ACE and effecting vasodilation comprising administering the nitrosothiol compounds of the invention to an animal.

CLM What is claimed is:

1. A pharmaceutical composition, comprising: (a) a therapeutically effective amount of a compound having the formula: ##STR20## wherein R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5, or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, phenyl, or C.sub.1 -C.sub.4 alkyl substituted by phenyl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl, or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and

R.sup.7 are methyl or C.sub.1 -C.sub.4 alkanoyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, phenoxy, or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.4 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 2; n is 0 to 2; and (b) a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein said compound is S-nitrosocaptopril.

3. A compound selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-(4-amino-2-S-nitroso)mercaptomethylbutanoyl)-L-proline, 1-[2-(S-nitroso)mercaptomethyl-6-(N-methyl-N-acetyl-amino)-hexanoyl]-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethylpentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso)mercaptomethylpentanoyl]-4hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethylpentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3-(S-nitroso)-mercaptomethyl-pentanoyl]-L-proline, and S-nitroso-L-cysteinyl-L-proline.

4. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 3 and a pharmaceutically acceptable carrier.

5. A method for treating a cardiovascular-renal disease, comprising administering to an animal in need of such treatment an effective amount of a compound having the formula: ##STR21## wherein R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5, or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, phenyl, or C.sub.1 -C.sub.4 alkyl substituted by phenyl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl, or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl or C.sub.1 -C.sub.4 alkanoyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, phenoxy, or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.4 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 2; and n is 0 to 2.

6. A method for treating a cardiovascular-renal disease, comprising administering to an animal in need of such treatment an effective amount of a compound selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-(4-amino-2-S-nitroso)mercapto-methylbutanoyl)-L-proline, 1-[2-hexanoyl]-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso)mercapto-methylpentanoyl]-4hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3-(S-nitroso)-mercaptomethylpentanoyl]-L-proline, and S-nitroso-L-cysteinyl-L-proline.

7. A method for treating **scleroderma**, comprising administering to an animal in need of such treatment a therapeutically effective amount of a compound having the formula: ##STR22## wherein R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5, or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, phenyl, or C.sub.1 -C.sub.4 alkyl substituted by phenyl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl, or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl or C.sub.1 -C.sub.4 alkanoyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, phenoxy, or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.4 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 2; and n is 0 to 2.

8. A method for treating **scleroderma**, comprising administering to an animal in need of such treatment a therapeutically effective amount of a compound selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-

cysteinyl-L-proline, 1-(4-amino-2-nitroso)mercapto-methylbutanoyl)-L-proline, 1-[2-(S-nitroso)mercaptomethyl-6-(N-methyl-N-acetyl-amino)-hexanoyl]-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethylpentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso)mercaptomethylpentanoyl]-4-hydroxy-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3-(S-nitroso)-mercaptomethylpentanoyl]-L-proline, and S-nitroso-L-cysteinyl-L-proline.

9. A method for treating toxemia of pregnancy, comprising administering to an animal in need of such treatment an effective amount of a compound having the formula: ##STR23## wherein R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5, or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, phenyl, or C.sub.1 -C.sub.4 are substituted by phenyl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl, or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl or C.sub.1 -C.sub.4 alkanoyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, phenoxy, or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.4 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 2; n is 0 to 2.

10. A method for treating toxemia or pregnancy, comprising administering to an animal in need of such treatment an effective amount of a compound selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-(4-amino-2-S-nitroso)mercapto-methylbutanoyl)-L-proline, 1-[2-(S-nitroso)mercaptomethyl-6-(N-methyl-N-acetyl-amino)-hexanoyl]-L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethylpentanoyl -L-proline, 1-[5-guanidino-2-(S-nitroso)mercaptomethyl-pentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3-(S-nitroso)-mercaptomethylpentanoyl]-L-proline, and S-nitroso-L-cysteinyl-L-proline.

11. A method of inhibiting angiotensin converting enzyme and effecting vasodilation in an animal comprising administering to the animal an effective amount of a compound having the formula: ##STR24## wherein R is hydroxy, NH.sub.2, NHR.sup.4, NR.sup.4 R.sup.5, or C.sub.1 -C.sub.7 alkoxy, wherein R.sup.4 and R.sup.5 are C.sub.1 -C.sub.4 alkyl, phenyl, or C.sub.1 -C.sub.4 alkyl substituted by phenyl; R.sup.1 is hydrogen, C.sub.1 -C.sub.7 alkyl, or C.sub.1 -C.sub.7 alkyl substituted by phenyl, amino, guanidino, NHR.sup.6 or NR.sup.6 R.sup.7, wherein R.sup.6 and R.sup.7 are methyl or C.sub.1 -C.sub.4 alkanoyl; R.sup.2 is hydrogen, hydroxy, C.sub.1 -C.sub.4 alkoxy, phenoxy, or C.sub.1 -C.sub.7 alkyl; R.sup.3 is hydrogen, C.sub.1 -C.sub.4 alkyl or C.sub.1 -C.sub.7 alkyl substituted by phenyl; m is 2; n is 0 to 2.

12. A method of inhibiting angiotensin converting enzyme and effecting vasodilation in an animal comprising administering to the animal a therapeutically effective amount of a compound selected from the group consisting of N-acetyl-S-nitroso-D-cysteinyl-L-proline, N-acetyl-S-nitroso-D,L-cysteinyl-L-proline, 1-4-amino-2-S-nitroso)mercaptomethylbutanoyl)-L-proline, 1-[2-(S-nitroso)mercaptomethyl-6-(N-methyl-N-acetyl-amino)-hexanoyl]-L-proline, 1-[5-guanidino -2-(S-nitroso) mercaptomethylpentanoyl]-L-proline, 1-[5-amino-2-(S-nitroso)mercaptomethylpentanoyl]-4-hydroxy-L-proline, 1-[-5-guanidino-2-(S-nitroso)mercaptomethylpentanoyl]-4-hydroxy-L-proline, 1-[2-aminomethyl-3-(S-nitroso)-mercaptomethyl-pentanoyl]-L-proline, and S-nitroso-L-cysteinyl-L-proline.

13. The method of any one of claims 5-12, wherein said compound is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

14. The method of claim 13, wherein said pharmaceutical composition comprises, per unit dose, one picomole to ten millimole of said

compound, which is administered one to four times daily.

15. The method of claim 13, wherein said pharmaceutical composition is int he form of an injectable solution which is administered by continuous intravenous infusion.

16. The method of any of claims 5-12, wherein said animal is a human.

17. The method of any one of claims 5, 7, 9 and 11, wherein said compound is S-nitrosocaptopril.

L6 ANSWER 16 OF 16 USPATFULL

ACCESSION NUMBER:	80:15087 USPATFULL		
TITLE:	Topical application of thioglycolic acid in the treatment of dermatological conditions		
INVENTOR(S):	Sheffner, Aaron L., 18 Trombley Dr., Livingston, NJ, United States 07039		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 4195095		19800325
APPLICATION INFO.:	US 1978-933506		19780814 (5)
DISCLAIMER DATE:	19970815		
RELATED APPLN. INFO.:	Division of Ser. No. US 1969-813750, filed on 4 Apr 1969, now patented, Pat. No. US 4107330		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Schenkman, Leonard		
LEGAL REPRESENTATIVE:	Cushman, Darby & Cushman		
NUMBER OF CLAIMS:	13		
EXEMPLARY CLAIM:	1		
LINE COUNT:	332		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A preparation containing as an active ingredient 0.1 to 10% by weight of thioglycolic acid and the salts, esters, and acid amides thereof, is employed in the treatment of fatty cysts, dandruff, **scleroderma** and other dermatological disorders.

CLM What is claimed is:

1. A process for the treatment of dandruff comprising applying to the dandruff effected skin of a person suffering therefrom, a dandruff alleviating amount of a topical composition containing about 0.1 to about 10% by weight of thioglycolic acid or a pharmaceutically acceptable salt thereof, and keeping said composition in contact with the affected skin for several hours.

2. The process of claim 1 wherein said concentration is 0.1% to 5% by weight.

3. The process of claim 1 wherein said concentration is 0.1 to 2% by weight.

4. The process of claim 1 wherein 0.1 to 5% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

5. The process of claim 1 wherein 0.1 to 2.0% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

6. The process of claim 1 wherein said thioglycolic acid is applied topically in the form of a pharmaceutically acceptable acid solution of the following composition:

Thioglycolic acid	5.0 g.
Hexachlorophene	1.0 g.
Disodium EDTA	0.05 g.

Sodium hydroxide qs	pH 7.0
Distilled water	100 ml.

7. The process of claim 1 wherein said thioglycolic acid is applied topically in the form of a pharmaceutically acceptable lotion of the following composition:

Thioglycolic acid	2.0 g.
Triethanolamine	to pH 7.0
Disodium EDTA	0.05 g.
Calamine	8.0 g.
Hexachlorophene	1.0 g.
Distilled water qs	100 ml.

8. A process for treating fatty cysts by softening the skin and increasing its permeability in a localized area comprising applying to the fatty cyst effected skin of a person suffering thereform, a fatty cyst alleviating amount of a topical composition containing from about 0.1 to about 10% by weight of thioglycolic acid or a pharmaceutically acceptable salt thereof, and keeping said composition in contact with the affected skin for several hours.

9. The process of claim 8 wherein 0.1 to 5% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

10. The process of claim 8 wherein 0.1 to 2.0% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

11. A process for treating **scleroderma** by softening the skin and increasing its permeability in a localized area comprising applying to the **scleroderma** effected skin of a person suffering therefrom, a **scleroderma** alleviating amount of a topical composition containing from about 0.1 to about 10% by weight of thioglycolic acid or a pharmaceutically acceptable salt thereof, and keeping said composition in contact with the affected skin for several hours.

12. The process of claim 11 wherein 0.1 to 5% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

13. The process of claim 11 wherein 0.1 to 2.0% by weight of the sodium salt of thioglycolic acid is applied topically to the skin.

L39 ANSWER 3 OF 9 USPATFULL
DETD

.mu.M IC.sub.50s on Published IC.sub.50s on
PDE10 from 786-O cloned PDE10
Inhibitor cGMP cAMP cGMP cAMP

IBMX 6.3 4.5 11 17
Zaprinast 17 13 14 22
Dipyridamole 0.58 0.37 0.45 1.2
E4021 3.2 2.8 4.2 7.2
Vinpocetine 45 38 73 77
cAMP 0.43 0.39
cGMP 5.2 14
EHNA 46 60 >100 >100
Milrinone 103 84 >100 >100
Rolipram 57 40 >100 >100

EHNA, Milrinone, and Rolipram are N = 1. Others are an average of N = 2.
DETD (A) Cyclooxygenase (**COX**) **Inhibition**
DETD
TABLE 1

COX I
EXAMPLE % **Inhibition**(100 .mu.M)

Sulindac sulfide 86
1 <25
DETD The advantage of very low **COX inhibition** is that compounds of this invention can be administered to patients without the side effects normally associated with **COX inhibition**

PI US 6538029 B1 20030325

Since the first report in 1958 of an enzymatic activity capable of hydrolyzing cAMP, it has become clear that this enzymatic activity, termed cyclic nucleotide phosphodiesterase (PDE), consists of a complex isozymic superfamily represented by different forms, of which more than thirty have been identified and cloned. These isozymic PDE forms have been grouped into seven broad gene families based upon similar structural and functional relationships: Ca.sup.2+ --calmodulin-dependent (PDE1), cyclic guanosine monophosphate, cGMP, stimulated (**PDE2**), cGMP inhibited (PDE3), cAMP specific (PDE4), cGMP specific (**PDE5**), photoreceptor (PDE6), and higher affinity drug-resistant cAMP specific (PDE7). A number of reviews have been written that describe the characteristics of these different PDE forms, their regulation, potential physiological function, and progress in development of pharmacological inhibitors of PDE as therapeutic agents. Gene family-specific inhibitors have been found for all but the PDE7 gene family, but no pharmacological inhibitor is yet capable of selectively inhibiting a specific PDE isoform within a given gene family. It is believed that selective elevation of cAMP levels in

L2 ANSWER 90 OF 92 USPATFULL

DETD A 1.8 kb region of PDE9A encoding the full length of the protein was cloned into the baculovirus transfer vector pFASTBAC, expressed in sf9 cells, and a cell lysate prepared from these cells for enzyme assays. FIG. 3 shows the kinetics of enzyme activity of recombinant, purified PDE9A with cGMP as a substrate. PDE9A has a very high affinity for cGMP with a Km of 170 nM, and a very low affinity for cAMP (Km=230 .upsilon.M, data not shown). FIG. 4 shows the dependence of PDE9A on divalent cations for maximal activity with a preference for Mn.sup.++ over Mg.sup.++ or Ca.sup.++. The effects of various known PDE inhibitors on the activity of PDE9A are shown in FIG. 5. PDE9A was not inhibited by up to 100 .mu.M of rolipram (inhibitor of PDE4), dipyridamole (inhibitor of **PDE2**, 4, 5, and 6), SKF94120 (inhibitor of PDE3), vinpocetine (inhibitor of PDE1), or IBMX (non-specific PDE inhibitor). PDE9A was inhibited by zaprinast (inhibitor of **PDE5** and 6) with an IC.sub.50 of 35 .mu.M. Membrane-based northern analysis shows the expression of this sequence in various tissues, with the most significant expression in testis, ovary, small intestine, and colon. Electronic northern analysis using the LIFESEQ database further shows the expression of this sequence in various tissues, at least 50% of which are cancerous and at least 25% of which involves inflammation or the immune response. Of particular note is the expression of PDE9A in Crohn's disease.

L2 ANSWER 88 OF 92 USPATFULL
DETD

Compound	log IC.sub.50, mol/l] **PDE5**	PDE4	PDE3	**PDE2**	PDE1
3		6.45	7.14		
4	5.45	7.54	6.67	4.80	<4
5		7.75	7.15		
11		7.85	7.23		
16.		7.96	6.73		
17		7.94	6.38		
18		7.87	6.74		
19		8.18	7.56		
21		7.67	6.34		
23		8.56	6.64		
24		8.51	7.64		

L2 ANSWER 78 OF 92 USPATFULL

SUMM [0004] A pharmaceutical product that provides a **PDE5** inhibitor is currently available, and is marketed under the trademark VIAGRA.RTM.. The active ingredient in VIAGRA.RTM. is sildenafil. The product is sold as an article of manufacture including 25, 50, and 100 mg tablets of sildenafil and a package insert. The package insert provides that sildenafil is a more potent inhibitor of **PDE5** than other known phosphodiesterases (greater than 80 fold for PDE1 inhibition, greater than 1,000 fold for **PDE2**, PDE3, and PDE4 inhibition). The IC.sub.50 for sildenafil against **PDE5** has been reported as 3 nM (Drugs of the Future, 22(2), pp. 128-143 (1997)), and as 3.9 nM (Boolell et al., Int. J. of Impotence Res., 8 p. 47-52 (1996)). Sildenafil is described as having a 4,000-fold selectivity for **PDE5** versus PDE3, and only a 10-fold selectivity for **PDE5** versus PDE6. Its relative lack of selectivity for PDE6 is theorized to be the basis for abnormalities related to color vision

PI US 2001053780 A1 20011220

L2 ANSWER 75 OF 92 USPATFULL

SUMM [0002] This invention relates to the use of one or more forms of phosphodiesterase type 2 ("**PDE2**") and phosphodiesterase type 5 ("**PDE5**") and/or protein kinase G to identify compounds useful for the treatment and prevention of pre-cancerous and cancerous lesions in mammals, and to pharmaceutical compositions containing such compounds, as well as to therapeutic methods of treating neoplasia with such compounds.

SUMM [0011] In the course of researching why some **PDE5** inhibitors singly induced apoptosis while others did not, we uncovered a form of cyclic GMP-specific phosphodiesterase activity, not previously described. This new phosphodiesterase activity was previously uncharacterized. . Without being limited to a specific theory, we believe this novel PDE activity may be a novel conformation of **PDE2** that substantially lacks cAMP-hydrolyzing activity, i.e. it is cGMP-specific. Classic **PDE2** is not cGMP-specific (it also hydrolyzes cAMP), classic **PDE2** is also found in neoplastic cells. This new PDE and **PDE2** are useful in screening pharmaceutical compounds for desirable anti-neoplastic properties. Basically, in neoplastic cells when **PDE5** and the **PDE2** activity (in its novel and conventional conformations) are inhibited by an anti-neoplastic **PDE5**-inhibiting compound, the result is apoptosis. When only **PDE5** is inhibited (but not the several forms of **PDE2**), apoptosis does not occur.

SUMM [0018] Another embodiment of this invention involves evaluating whether a compound causes an increase in cGMP-dependent protein kinase G ("PKG") activity and/or a decrease of .beta.-catenin in neoplastic cells. It has been found that unexpected characteristics of SAANDs include the elevation of PKG activity and a decrease in .beta.-catenin in neoplastic cells exposed to a SAAND. We believe that the elevation of PKG activity is due at least in part by the increase in cGMP caused by SAANDs inhibition of the appropriate PDEs, as described above. The other characteristics of SAANDs are (1) inhibition of **PDE5** as reported in the '694 patent above, (2) inhibition of the novel cGMP-specific PDE conformation, (3) inhibition of **PDE2**; (4) the fact that they increase intracellular cGMP in neoplastic cells, and (5) the fact that they decrease cAMP levels in some types of neoplastic cells.

SUMM [0019] Thus, one embodiment of the novel method of this invention is evaluating whether a compound causes PKG activity to elevate in neoplastic cells and whether that compound inhibits **PDE5**. Another embodiment of the novel screening method of this invention is evaluating whether a compound that causes PKG activity to elevate in neoplastic cells and whether that compound inhibits the novel cGMP-specific PDE described above and/or **PDE2**. Still a third embodiment is evaluating whether a compound causes PKG activity to elevate in neoplastic cells and whether that compound causes cGMP to rise in neoplastic cells and/or causes cAMP levels to fall. Compounds successfully evaluated in such fashions have application as SAANDs.

DETD [0092] The novel PDE of this invention and **PDE2** are useful with or without **PDE5** to identify compounds that can be used to treat or prevent neoplasms, and that are not characterized by serious side effects.

DETD [0105] Obviously, a compound that exhibits a lower COX-I or COX-2 inhibitory activity in relation to its greater combined **PDE5** /novel PDE/**PDE2** inhibitory activities may be a desirable compound.

DETD [0108] Compounds can be screened for inhibitory effect on the activity of the novel phosphodiesterase of this invention using either the enzyme

isolated as described above, a recombinant version, or using the novel PDE and/or **PDE2** together with **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by RIA and compared to untreated and zaprinast-treated cells.

PI US 2002009764 A1 20020124

L2 ANSWER 73 OF 92 USPATFULL

SUMM The amino acid sequences of all mammalian PDEs identified to date include a highly conserved region of approximately 270 amino acids located in the carboxy terminal half of the protein [Charbonneau, et al., Proc. Natl. Acad. Sci. (USA) 83:9308-9312 (1986)]. The conserved domain includes the catalytic site for cAMP and/or cGMP hydrolysis and two putative zinc binding sites as well as family specific determinants [Beavo, Physiol. Rev. 75:725-748 (1995); Francis, et al., J. Biol. Chem. 269:22477-22480 (1994)]. The amino terminal regions of the various PDEs are highly variable and include other family specific determinants such as: (i) calmodulin binding sites (PDE1); (ii) non-catalytic cyclic GMP binding sites (**PDE2**, **PDE5**, PDE6); (iii) membrane targeting sites (PDE4); (iv) hydrophobic membrane association sites (PDE3); and (v) phosphorylation sites for either the calmodulin-dependent kinase II (PDE1), the cAMP-dependent kinase (PDE1, PDE3, PDE4), or the cGMP dependent kinase (**PDE5**) [Beavo, Physiol. Rev. 75:725-748 (1995); Manganiello, et al., Arch. Biochem. Acta 322:1-13 (1995); Conti, et al., Physiol. Rev. 75:723-748 (1995)].

SUMM Members of the PDE1 family are activated by calcium-calmodulin. Three genes have been identified; PDE1A and PDE1B preferentially hydrolyze cGMP while PDE1C has been shown to exhibit a high affinity for both cAMP and cGMP. The **PDE2** family is characterized as being specifically stimulated by cGMP [Loughney and Ferguson, supra]. Only one gene has been identified, PDE2A, the enzyme product of which is specifically inhibited by erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). Enzymes in the PDE3 family are specifically inhibited by cGMP. Two genes are known, PDE3A and PDE3B, both having high affinity for both cAMP and cGMP, although the V.sub.max for cGMP hydrolysis is low enough that cGMP functions as a competitive inhibitor for cAMP hydrolysis. PDE3 enzymes are specifically inhibited by milrinone and enoximone [Loughney and Ferguson, supra]. The PDE4 family effects cAMP hydrolysis and includes four genes, PDE4A, PDE4B, PDE4C, and PDE4D, each having multiple splice variants. Members of this family are specifically inhibited by the anti-depressant drug rolipram. Members of **PDE5** family bind cGMP at non-catalytic sites and preferentially hydrolyze cGMP. Only one gene, PDE5A, has been identified. The photoreceptor PDE6 enzymes specifically hydrolyze cGMP [Loughney and Ferguson, supra]. Genes include PDE6A and PDE6B (the protein products of which dimerize and bind two copies of a smaller .gamma. inhibitory subunit to form rod PDE), in addition to PDE6C which associates with three smaller proteins to form cone PDE. The PDE7 family effects cAMP hydrolysis but, in contrast to the PDE4 family, is not inhibited by rolipram [Loughney and Ferguson, supra]. Only one gene, PDE7A, has been identified. The PDE8 family has been shown to hydrolyze both cAMP and cGMP and is insensitive to inhibitors specific for PDEs 1-5. Depending on nomenclature used, PDE8 is also referred to as PDE10, but is distinct from PDE10 described herein. The PDE9 family preferentially hydrolyzes cAMP and is not sensitive to inhibition by rolipram, a PDE4-specific inhibitor, or isobutyl methyl xanthine (IBMX), a non-specific PDE inhibitor. Depending on nomenclature used, PDE9 is also referred to as PDE8, but is distinct from PDE8 mentioned above. To date, two genes have been identified in the PDE9 family.

DETD

TABLE 1

PDE10 Inhibition with Isozyme-specific PDE Inhibitors

Inhibitor	Target PDE10 Family	Target PDE10 IC.sub.50 (.mu.M)	IC.sub.50 (.mu.M)
SCH46642	PDE1	14	0.2.sup.5
EHNA	**PDE2**	477	0.8.sup.2
Cilostamide	PDE3	100	0.04-0.9.sup.3

Rolipram PDE4 529 0.18-0.5.sup.4
DMPPO **PDE5** 9 0.003.sup.1
IBMX non-specific 59 2-20.sup.1

.sup.1Coste and Grodin, Biochem. Pharmacol. 50:1577-1585 (1995).
.sup.2Podzuweit, et al. Cell. Signaling 7:733-738 (1995)
.sup.3Manganiello et al., in Isoenzymes of Cyclic Nucleotide Phosphodiesterases, Beavo and Housley (Eds.), John Wiley and Sons, Ltd., pp. 87-116 (1990)
.sup.4Bolger et al., Mol. Cell. Biol. 13:6558-6571 (1993)
.sup.5Ahn, et al., Abstract from the 9th International Conference on Second Messengers and Phosphoproteins, Nashville, TN, 1995, p. 86.

PI US 6350603 B1 20020226

L2 ANSWER 66 OF 92 USPATFULL
DETD [0248] 35) One or more of a PDE inhibitor, more particularly a PDE 2, 3, 4, 5, 7 or 8 inhibitor, preferably **PDE2** or **PDE5** inhibitor and most preferably a **PDE5** inhibitor (see hereinafter), said inhibitors preferably having an IC50 against the respective enzyme of less than 100 nM. Suitable cGMP **PDE5** inhibitors for the use according to the present invention include:
PI US 2002052370 A1 20020502

L2 ANSWER 65 OF 92 USPATFULL

SUMM [0144] PDE inhibitors such as **PDE2** (e.g. erythro-9-(2-hydroxyl-3-nonyl)-adenine) and Example 100 of EP 0771799-incorporated herein by reference) and in particular a **PDE5** inhibitor (e.g. sildenafil, 1-{[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-trazin-2-yl)-4-ethoxyphenyl]sulfonyl}-4-ethylpiperazine i.e. vardenafil / Bayer BA 38-9456) and IC351 (see structure below, Icos Lilly). ##STR10##

PI US 2002052395 A1 20020502
US 6448293 B2 20020910

L2 ANSWER 59 OF 92 USPATFULL

DETD In particular, a compound of the present invention, i.e., Sample 66, has an IC.sub.50 vs. human recombinant PDE4B of 0.015 .mu.M, but has an IC.sub.50 vs. PDE1A of 80 .mu.M, vs. PDE1B of 100 .mu.M, vs. PDE1C of 12 .mu.M, vs. **PDE2** of 450 .mu.M, vs. PDE3A of 40 .mu.M, vs. **PDE5** of 270 .mu.M, and vs. PDE7 of 36 .mu.M. This illustrates the selectivity of the present compound with respect to inhibiting PDE4.

DETD Recombinant production of human PDE1B, **PDE2**, PDE4A, PDE4B, PDE4C, PDE4D, **PDE5**, and PDE7 was carried out similarly to that described in Example 7 of U.S. Pat. No. 5,702,936, incorporated herein by reference, except that the yeast transformation vector employed, which is derived from the basic ADH2 plasmid described in Price et al., Methods in Enzymology, 185, pp. 308-318 (1990), incorporated yeast ADH2 promoter and terminator sequences and the Saccharomyces cerevisiae host was the protease-deficient strain BJ2-54 deposited on Aug. 31, 1998 with the American Type Culture Collection, Manassas, Virginia, under accession number ATCC 74465. Transformed host cells were grown in 2.times.SC-leu medium, pH 6.2, with trace metals, and vitamins. After 24 hours, YEP medium-containing glycerol was added to a final concentration of 2X YET/3% glycerol. Approximately 24 hr later, cells were harvested, washed, and stored at -70.degree. C.

PI US 6423710 B1 20020723

L2 ANSWER 55 OF 92 USPATFULL

SUMM A pharmaceutical product, which provides a **PDE5** inhibitor, is currently available and marketed under the trademark VIAGRA.RTM.. The active ingredient in VIAGRA.RTM. is sildenafil. The product is sold as an article of manufacture including 25, 50, and 100 mg tablets of sildenafil and a package insert. The package insert provides that sildenafil is a more potent inhibitor of **PDE5** than other known phosphodiesterases (greater than 80 fold for PDE1 inhibition, greater than 1,000 fold for **PDE2**, PDE3, and PDE4 inhibition). The IC.sub.50 for sildenafil against **PDE5** has been reported as 3 nM (Drugs of the Future, 22(2), pp. 128-143 (1997)), and as 3.9 nM (Boolell et al., Int. J. of Impotence Res., 8 p. 47-52 (1996)). N.C. Sildenafil is described as having a 4,000-fold selectivity for **PDE5** versus PDE3, and only a 10-fold selectivity for **PDE5** versus PDE6. Its relative lack of selectivity for PDE6 is theorized to be the basis for abnormalities related to color vision.

PI US 6451807 B1 20020917

L2 ANSWER 47 OF 92 USPATFULL
DETD [0120] The results of the above PDE assays are presented in the following table. The results indicate that the (+) enantiomer of flosequinan demonstrated more PDE1 and PDE3 inhibitory activity when compared with the (-) enantiomer of flosequinan. These empirical results could not be predicted.

Target Phosphodiesterase	% Inhibition w/ 100 .mu.M racemic mixture of flosequinan	% Inhibition w/ 100 .mu.M (+)-flosequinan	% Inhibition w/ 100 .mu.M (-)-flosequinan
PDE1	31	28	11
PDE2	18	18	13
PDE3	26	32	5
PDE4	24	6	1
PDE5	11	17	10
PDE6	21	22	21

PI US 2002147217 A1 20021010

L2 ANSWER 48 OF 92 USPATFULL
DETD [0137] The results of the above PDE assays are presented in the following table. The (+) enantiomer of flosequinan demonstrated more PDE1 and PDE3 inhibitory activity when compared with the (-) enantiomer of flosequinan. These empirical results could not be predicted.

Target Phosphodiesterase	% Inhibition w/ 100 .mu.M racemic mixture of flosequinan	% Inhibition w/ 100 .mu.M (+)-flosequinan	% Inhibition w/ 100 .mu.M (-)-flosequinan
PDE1	31	28	11
PDE2	18	18	13
PDE3	26	32	5
PDE4	24	6	1
PDE5	11	17	10
PDE6	21	22	21

PI US 2002143031 A1 20021003
US 6562838 B2 20030513

L2 ANSWER 49 OF 92 USPATFULL
DETD
TABLE 12

PDE ISOZYME SPECIFICITY
% inhibition by 100 .mu.M test

Compound number	PDE Isozyme	compound 1-25	25-50	50-75	75-100
12	PDE1			X	
	PDE2			X	
	PDE3			X	
	PDE4			X	
	PDE5			X	
43	PDE1			X	
	PDE2			X	
	PDE3			X	
	PDE4			X	
	PDE5			X	
	PDE1	X			
	PDE2	X			

```
 PDE3  X
 PDE4    X
   PDE5 X
136  PDE1   X
   PDE2   X
 PDE3    X
 PDE4    X
   PDE5 X
PI     US 6458829         B1   20021001
       WO 2000014083  20000316
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SUMM This invention represents a novel therapy for treating cystic fibrosis patients without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 2 ("**PDE2**") that also preferably inhibits phosphodiesterase 5 ("**PDE5**") to a mammal in need of treatment for cystic fibrosis. In narrower aspects of this invention, this invention involves the administration of compounds of Formula I below to a mammal in need of treatment for cystic fibrosis.

DRWD FIG. 1 is a graph that compares the **PDE2** and **PDE5** mRNA levels in control and activated macrophages.

DETD As discussed above, the present invention includes the administration of an inhibitor of **PDE2** to a mammal in need of treatment for cystic fibrosis. Preferably, the compound also inhibits **PDE5**. In addition, this invention includes the use of compounds of Formula I below (as well as their pharmaceutically acceptable salts) for treating a mammal with cystic fibrosis: ##STR1##

DETD Compounds of this invention are inhibitors of phosphodiesterases **PDE2**. For convenience, the PDE inhibitory activity of such compounds can be tested as taught in U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998 to Pamukcu et al., which is incorporated herein by reference. Thus, compounds employed in this invention are useful inhibitors of **PDE2** and preferably also **PDE5**. Most preferably, such compounds have an IC.sub.50 for **PDE2** of no more than 25 .mu.M.

DETD Additional compounds besides those of Formula I can be identified for inhibitory effect on the activity of **PDE2** and/or **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by radioimmunoassay ("RIA") and compared to untreated and drug-reated tissue samples and/or isolated enzymes.

DETD Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for the PDE enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H--cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/mL BSA) is mixed with the drug to be tested in a total volume of iu 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with isolated **PDE2** and/or **PDE5**. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/mL snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 minutes at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 mL of 100% methanol. Assay samples are applied to 1 mL Dowex 1-X8 column; and washed with 1 mL of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the column is combined and measured with a scintillation counter. The degree of phosphodiesterase inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound but with drug solvent).

DETD In addition to compounds disclosed herein, other compounds that inhibit both **PDE2** and **PDE5** include compounds disclosed in U.S. Pat. No. 5,401,774 (e.g., exisulind), U.S. Pat. Nos. 6,063,818, 5,998,477, and 5,965,619. These patents are incorporated herein by reference. Preferable compounds include those having a **PDE2** IC.sub.50 less than about 25 .mu.M.

DETD When referring to an "a physiologically effective amount of an inhibitor of **PDE2** and **PDE5**" we mean not only a single compound that inhibits those enzymes but a combination of several compounds, each of which can inhibit one or both of those enzymes. Single compounds that

inhibit both enzymes are preferred.

DETD When referring to an "inhibitor [that] does not substantially inhibit COX I or COX II," we mean that in the ordinary sense of the term. By way of example only, if the inhibitor has an IC.sub.50 for either **PDE2** or **PDE5** that is at least half of the IC.sub.50 of COXI and/or COXII, a drug achieving the PDE IC.sub.50 in the blood could be said not to substantially inhibit the COX enzymes. Preferably, the IC.sub.50 for the COX enzymes is in the order of 10 fold or more higher than the IC.sub.50 for **PDE2**/**PDE5**. Preferably the IC.sub.50 for each of the COX enzymes is greater than about 40 .mu.M.

DETD As to identifying structurally additional **PDE2** and **PDE5** inhibiting compounds besides those of Formula I that can be effective therapeutically for CYSTIC FIBROSIS, one skilled in the art has a number of useful model compounds disclosed herein (as well as their analogs) that can be used as the bases for computer modeling of additional compounds having the same conformations but different chemically. For example, software such as that sold by Molecular Simulations Inc. release of WebLab.RTM. ViewerPro.TM. includes molecular visualization and chemical communication capabilities. Such software includes functionality, including 3D visualization of known active compounds to validate sketched or imported chemical structures for accuracy. In addition, the software allows structures to be superimposed based on user-defined features, and the user can measure distances, angles, or dihedrals.

DETD To further assist in identifying compounds that can be screened and then selected using the criterion of this invention, knowing the binding of selected compounds to **PDE5** and **PDE2** protein is of interest. By the procedures discussed below, it is believed that that preferable, desirable compounds meeting the selection criteria of this invention bind to the cGMP catalytic regions of **PDE2** and **PDE5**.

DETD Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l . The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter. The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for **PDE5** of 4 .mu.M, an IC.sub.50 value for PDE1 of 3 .mu.M, and an IC.sub.50 value for PDE4 of 6 .mu.M.

DETD As demonstrated below, we found that macrophages contain **PDE2** and **PDE5**. The inhibition of **PDE2** particularly with lung epithelial cells and **PDE5** inhibition leads to apoptosis of macrophage cells. Inhibition of PDEs in epithelial cells would result in cGMP accumulation, resulting in correction of ion transport defect in these cells. We believe the administration of a **PDE2** inhibitor can treat the progression of cystic fibrosis, particularly when **PDE5** is also inhibited.

DETD ii. **PDE2** and **PDE5** mRNA Levels in Treated and Untreated U937 Cells by RT-PCR

DETD U937 cells (from ATCC Rockville, Md.) were grown in RPMI media supplemented with 5% FCS, glutamine, antibiotic/antimycotic and sodium pyruvate. Total RNA was isolated from two U937 cultures, one treated with 5nM TPA for 48 hours and one grown in normal media as listed above, using the Rouche High Pure RNA Isolation Kit (cat# 1 828 665) as per manufacturers protocol. cDNA was then synthesized from the total RNA using GibcoBRL Superscriptll (Cat # 18064-022) reverse transcriptase as per manufacturers protocol. The resulting cDNA was used as a template for RT-PCR reactions using primer sets specific for **PDE2** (forward: CCCAAAGTGGAGACTGTCTACACCTAC, reverse: CCGGTTGTCTTCCAGCGTGTC) or **PDE5** (forward: GGGACTTTACCTTCTCATAC, reverse: GTGACATCCAAATGACTAGA). mRNA for **PDE2** and 5 were both present in the untreated U937 cells. Upon treatment with TPA, the relative amounts of **PDE2** mRNA increased 5 fold. Therefore, U937 cells treated with TPA and driven to differentiate into an activated macrophage like state have elevated levels of **PDE2** mRNA (see FIG. 1).

DETD iii. Confirmation of **PDE2** and **PDE5** Protein Within U937 Cells by Indirect Immunofluorescence

DETD The presence of **PDE2** and **PDE5** protein within U937 cells was confirmed by indirect immunofluorescence (IIF). U937 cells were cultured as above. Two U937 cultures, one grown in the presence of 5 nM TPA for 48 hours and one grown in normal media were processed. All cultures were collected by centrifugation (Shandon Cytospin, 2 minutes @ 600 rpm) onto poly-L lysine-coated slides and immediately fixed in fresh 3% paraformaldehyde buffered in PBS for 10 minutes. Adherent cultures were grown on coverslips and fixed as above. Cells were permeablized in 0.2% triton-100 for 2 minutes. Slides were blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in PBS) for 1 hour at room temperature.

DETD Slides were then incubated for 1 hour at 37.degree. C. in a humid chamber with antibodies specific for **PDE2** (generated in a sheep against the peptide TLAFQKEQKLKCECQA) or **PDE5** (generated in sheep against the peptide CAQLYETSLLENKRNQV). The **PDE5** antibody was used at a dilution of 1:200 and the **PDE2** antibody was used at a dilution of 1:100. All dilutions were performed in blocking buffer. Slides were then washed 2.times. for 10 minutes each in PBS and then incubated with a Cy3 conjugated secondary antibody (Jackson lmnunoResearch laboratories, Inc. Cat. # 713-166-147) diluted 1:1000 in blocking buffer, for 1 hour at 37.degree. C. in a humid chamber. Slides were then washed 2.times. for 10 minutes each in PBS and counterstained with DAPI (5 ng/ml) and mounted in VectaShield. Digital images were then obtained using a SPOT-2 camera and an Olympus IX-70 fluorescent microscope. Both **PDE2** and **PDE5** are present in the cytoplasm of U937 cells. There is an increase in the level of both **PDE2** and **PDE5** in TPA-treated U937 cells. These increased protein levels are seen in discrete perinuclear foci (see FIGS. 2 through 5).

DETD Cyclic GMP PDE levels in protein lysates extracted from TPA-treated and untreated U937 cells were also analyzed as follows. Cells were resuspended in 20 mM TRIS-HCl, 5 mM MgCl2, 0.5% Triton X-100, 0.1 mM EDTA, 10 mM benzamidine, 10 .mu.M TLCK, 20 nM aprotinin, 2 .mu.M leupeptin, 2 .mu.M pepstatin A, pH 8.0 were added. The cells were homogenized using a glass tissue grinder and teflon pestle. Samples were

ultracentrifuged at 100,000 .times. g for 1 hr at 0.degree. C. Supernatants were assayed at 0.25 .mu.M cGMP using the method from Thompson, W. J. et. al. Adv. Cyclic Nucleotide Res., 10: 69-92, 1979. Again, the level of cGMP hydrolytic activity increased upon TPA treatment/activation, compared with no treatment/unactivation (see FIG. 7). Both of these experiments corroborate the results of our experiments above that show that both cGMP **PDE2** and **PDE5** protein levels increase in U937 cells treated with TPA.

DETD The activity of specific PDE inhibitors contrast with the activity of compound 38 in U937 cells. By "specific" in this context, we mean the other PDE inhibitors that inhibited one PDE primarily, but not several PDEs (e.g., inhibiting **PDE2** and **PDE5** at roughly the same concentration). An example is sildenafil, which primarily inhibits **PDE5**, and only at much higher concentrations may only marginally inhibit other PDEs. Another example is rolipram (PDE4-specific).

DETD U937 cells were incubated in the presence of 0.3nM sildenafil or 0.5uM rolipram for 24 hours using the culture conditions described above. The cells were harvested and processed for IIF as described above using an antibody that specifically recognizes active caspase 3. Digital images are shown in FIGS. 10 and 11. No increase in the levels of apoptosis compared to normal background was observed. Therefore, the inhibition of only PDE4 or **PDE5** alone (i.e. without the inhibition of **PDE2**) is not sufficient to induce apoptosis in U937 cells.

DETD **PDE2** inhibition in these cells together with **PDE5** inhibition will cause an elevation of cGMP. The elevation of cGMP, in turn, corrects the defect the defect in ion (e.g., chloride) channel transport. Ion transport is necessary for in normal mucin excretion. Correcting this by causing cGMP elevation in cells with defective ion transport will restore normal mucin secretion response. Thus, **PDE2** and **PDE5** protein expression in pulmonary epithelial cells, specifically type II pneumocytes, from patients diagnosed with cystic fibrosis can be clinically treated with Compound 38, a **PDE2**/5 inhibitor.

CLM What is claimed is:

3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

33. The method of claim 32 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

PI US 6465494 B1 20021015

anti-human **PDE2**(1), diluted 1:1500 and affinity purified sheep anti-human **PDE5**(1), diluted 1:2000) and were incubated overnight at 4.degree. C. in a humid chamber. Sections were washed three times for five minutes each in phosphate buffered saline. Sections were incubated with a secondary antibody (diluted donkey anti-sheep biotin conjugated immunoabsorbed antibody 1:2500) in blocking buffer, Jackson lmmunoResearch Catalog #713-065-147. Sections were then incubated at room temperature for 30 minutes, washed three times in phosphate buffered saline.for 5 minutes. Diluted Vector ABC reagents were used as according to manufacture's protocol (vector Catalog #6106) and were incubated for 30 minutes at room temperature. Washed in phosphate buffered saline for 5 minutes. A DAB substrate kit was used per manufacture's protocol (Vector Catalog #SK-4100). Slides containing tissue were rinsed with distilled water and immediately immerse in Meyers hematoxylin for 1-5 minutes. Excess hematoxylin was rinsed off with distilled water. Slides were dipped 10 times in 2% glacial acetic acid solution followed by 10 dips in distilled water. Slides were incubated in bluing solution (15 mM ammonium water) 15 dips followed by 10 dips in water and dehydrated through an ethanol series. The labelled slides were then mounted in Permount (Sigma).

DETD Quantitative **PDE2** and **PDE5** Immunohistochemistry

DETD Rat pancreatic tissues on microscope slides were analyzed by the Automated Cellular Imaging System (Chromavision, Capistrano, Calif.) using the generic DAB application (rev. A). Each tissue specimen was thoroughly scanned and 10 islet regions were examined and areas of necrosis were avoided. Percentage of cells with positive signal for **PDE2** and **PDE5** was computed. See table below for generated values (Table I under results section).

DETD With these results, one aspect of this invention is a medical therapy that involves the administration of the **PDE2** inhibitor (preferably also a **PDE5** inhibitor) at the stage of Type I diabetes development when macrophage invasion is occurring. Macrophages release cytokines that can recruit other inflammatory cells such as lymphocytes and neutrophils, all of which can cause damage to islet cells. Thus, it is desirable to begin therapy at the stage of macrophage activation.

DETD

TABLE I

Quantitative Immunohistochemistry of **PDE2** and **PDE5** in Islet Cell Region

Tissue Identification	Stage of Disease	Mean Number of Cells with Brown Intensity in Islet (**PDE2**)	Mean Number of Cells with Brown Intensity in Islet (**PDE5**)
B14A	Chronic Type-I	85 .+-. 32.9	111 .+-. 14.5
B14D	B14A age-matched resistant control	71 .+-. 32.6	87 .+-. 7.2
B14B	Acute Type-I	56 .+-. 26.5	96 .+-. 6.8
B14E	B14B age-matched resistant control	78 .+-. 6.3	102 .+-. 8.5
B14C	Prone Type-I	58 .+-. 33.2	87 .+-. 6.4
B14F	B14C age-matched resistant control	57 .+-. 39.8	93 .+-. 4.9

DETD Human formalin-fixed paraffin-embedded 5-.mu.m thick pancreatic tissue was obtained from a 22 year-old female and another female of unknown age. Both patients had a history of Type-I diabetes mellitus. A serial dilution study demonstrated the optimal signal-to-noise ratio was 1:100 and 1:200 (**PDE2**), 1:500 and 1:1000 (**PDE5**). Anti-**PDE2** and anti-**PDE5** antibodies were used as the primary antibodies, and the principal detection system consisted of a Vector anti-sheep secondary (BA-6000) and Vector ABC-AP Kit (AK-5000) with a

Vector Red substrate kit (SK-5100), which was used to produce a fuchsia-colored red deposit. Tissues were also stained with a positive control antibody (CD31) to ensure the tissue antigens were preserved and accessible for immunohistochemical analysis. CD31 is present in monocytes, macrophages, granulocytes, B lymphocytes and platelets. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged using a DVC Digital Photo Camera coupled to a Nikon microscope.

DETD Human pancreatic tissue samples (1 and 2) exhibited positive staining for **PDE2** and **PDE5** proteins and immunostaining was mostly localized to pancreatic islet cells. In FIGS. 17a and 17b, pancreatic tissue specimens from the two human patients described above are stained for **PDE2** protein. In FIGS. 18a and 18b pancreatic tissue specimens from the two human patients described above are stained for **PDE5** protein.

CLM What is claimed is:

3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

34. The method of claim 33 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

PI. US 6479493 B1 20021112

MRNA levels in control and activated macrophages.

DETD Compounds of this invention are inhibitors of phosphodiesterases **PDE2**. For convenience, the PDE inhibitory activity of such compounds can be tested as taught in U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998 to Pamukcu et al., which is incorporated herein by reference. Thus, compounds employed in this invention are useful inhibitors of **PDE2** and preferably also **PDE5**. Most preferably, such compounds have an IC50 for **PDE2** of no more than 25 .mu.M.

DETD Additional compounds besides those of Formula I can be identified for inhibitory effect on the activity of **PDE2** and/or **PDE5** . Alternatively, cyclic nucleotide levels in whole cells are measured by radioimmunoassay ("RIX") and compared to untreated and drug-treated tissue samples and/or isolated enzymes.

DETD Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for the PDE enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/mL BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with isolated **PDE2** and/or **PDE5**. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/mL snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 minutes at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 mL of 100% methanol. Assay samples are applied to 1 mL Dowex 1-X8 column; and washed with 1 mL of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the column is combined and measured with a scintillation counter. The degree of phosphodiesterase inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound but with drug solvent).

DETD In addition to compounds disclosed herein, other compounds that inhibit both **PDE2** and **PDE5** include compounds disclosed in U.S. Pat. Nos. 5,401,774 (e.g., exisulind), 6,063,818, 5,998,477, and 5,965,619. These patents are incorporated herein by reference.

DETD When referring to an "a physiologically effective amount of an inhibitor of **PDE2** and **PDE5**" we mean not only a single compound that inhibits those enzymes but a combination of several compounds, each of which can inhibit one or both of those enzymes. Single compounds that inhibit both enzymes are preferred.

DETD When referring to an "inhibitor [that] does not substantially inhibit COX I or COX II," we mean that in the ordinary sense of the term. By way of example only, if the inhibitor has an IC.sub.50 for either **PDE2** or **PDE5** that is at least half of the IC.sub.50 of COXI and/or COXII, a drug achieving the PDE IC.sub.50 in the blood could be said not to substantially inhibit the COX enzymes. Preferably, the IC.sub.50 for the COX enzymes is in the order of 10 fold or more higher than the IC.sub.50 for **PDE2**/**PDE5**. Preferably, the IC.sub.50 for the COX enzymes is greater than about 40 .mu.M.

DETD As to identifying structurally additional **PDE2** and **PDE5** inhibiting compounds besides those of Formula I that can be effective therapeutically for type I diabetes, one skilled in the art has a number of useful model compounds disclosed herein (as well as their analogs) that can be used as the bases for computer modeling of additional compounds having the same conformations but different chemically. For example, software such as that sold by Molecular Simulations Inc. release of WebLab.RTM. ViewerPro.TM. includes molecular visualization and chemical communication capabilities. Such software includes functionality, including 3D visualization of known active

compounds to validate sketched or imported chemical structures for accuracy. In addition, the software allows structures to be superimposed based on user-defined features, and the user can measure distances, angles, or dihedrals.

DETD To further assist in identifying compounds that can be screened and then selected using the criterion of this invention, knowing the binding of selected compounds to **PDE5** and **PDE2** protein is of interest. By the procedures discussed below, it is believed that preferable, desirable compounds meeting the selection criteria of this invention bind to the cGMP catalytic regions of **PDE2** and **PDE5**.

DETD Examples of compounds that inhibit **PDE2** and **PDE5** (with insubstantial COX inhibition) include exisulind and compounds disclosed in U.S. Pat. Nos. 5,965,619 and 6,063,818 which are incorporated herein by reference.

DETD Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigmna) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter. The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for **PDE5** of 4 .mu.M, an IC.sub.50 value for PDE1 of 3 .mu.M, and an IC.sub.50 value for PDE4 of 6 .mu.M.

DETD As demonstrated below, we found that macrophages contain **PDE2** and **PDE5**, and the inhibition of **PDE2** particularly with **PDE5** inhibition leads to apoptosis of macrophage cells. We believe the administration of a **PDE2** inhibitor can treat the progression of Type I diabetes, particularly when **PDE5** is also inhibited.

DETD ii. **PDE2** and **PDE5** MRNA Levels in Treated and Untreated U937 Cells by RT-pCR

DETD U937 cells (from ATCC Rockville, Md.) were grown in RPMI media supplemented with 5% FCS, glutamine, antibiotic/antimycotic and sodium pyruvate. Total RNA was isolated from two U937 cultures, one treated with 5 nM TPA for 48 hours and one grown in normal media as listed above, using the Rouche High Pure RNA Isolation Kit (cat #1 828 665) as per manufacturers protocol. cDNA was then synthesized from the total RNA using GibcoBRL SuperscriptII (Cat #18064-022) reverse transcriptase as per manufacturers protocol. The resulting cDNA was used as a template

for RT-PCR reactions using primer sets specific for **PDE2** (forward: CCCAAAGTGGAGACTGTCTACACCTAC, reverse: CCGGTTGTCTTCCAGCGTGTC) or **PDE5** (forward: GGGACTTTACCTTCTCATAC, reverse: GTGACATCCAAATGACTAGA). mRNA for **PDE2** and 5 were both present in the untreated U937 cells. Upon treatment with TPA, the relative amounts of **PDE2** mRNA increased 5 fold. Therefore, U937 cells treated with TPA and driven to differentiate into an activated macrophage like state have elevated levels of **PDE2** mRNA (see FIG. 1).

DETD iii. Confirmation of **PDE2** and **PDE5** Protein Within U937 Cells by Indirect Immunofluorescence

DETD The presence of **PDE2** and **PDE5** protein within U937 cells was confirmed by indirect immunofluorescence (IIF). U937 cells were cultured as above. Two U937 cultures, one grown in the presence of 5 nM TPA for 48 hours and one grown in normal media were processed. All cultures were collected by centrifugation (Shandon Cytospin, 2 minutes @600 rpm) onto poly-L lysine-coated slides and immediately fixed in fresh 3% paraformaldehyde buffered in PBS for 10 minutes. Adherent cultures were grown on coverslips and fixed as above. Cells were permeablized in 0.2% Triton-100 for 2 minutes. Slides were blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in PBS) for 1 hour at room temperature.

DETD Slides were then incubated for 1 hour at 37.degree. C. in a humid chamber with antibodies specific for **PDE2** (generated in a sheep against the peptide TLAFQKEQKLKCECQA) or **PDE5** (generated in sheep against the peptide CAQLYETSLLENKRNQV). The **PDE5** antibody was used at a dilution of 1:200 and the **PDE2** antibody was used at a dilution of 1:100. All dilutions were performed in blocking buffer. Slides were then washed 2.times. for 10 minutes each in PBS and then incubated with a Cy3 conjugated secondary antibody (Jackson ImmunoResearch laboratories, Inc. Cat. #713-166-147) diluted 1:1000 in blocking buffer, for 1 hour at 37.degree. C. in a humid chamber. Slides were then washed 2.times. for 10 minutes each in PBS and counterstained with DAPI (5 ng/ml) and mounted in VectaShield. Digital images were then obtained using a SPOT-2 camera and an Olympus IX-70 fluorescent microscope. Both **PDE2** and **PDE5** are present in the cytoplasm of U937 cells. There is an increase in the level of both **PDE2** and **PDE5** in TPA-treated U937 cells. These increased protein levels are seen in discrete perinuclear foci (see FIGS. 2 through 5).

DETD cGMP hydrolysis levels in protein lysates extracted from TPA-treated and untreated U937 cells were also analyzed as follows. Cells were resuspended in 20 mM TRIS-HCl, 5 mM MgCl2, 0.5% Triton X-100, 0.1 mM EDTA, 10 mM benzamidine, 10 .mu.M TLCK, 20 nM aprotinin, 2 .mu.M leupeptin, 2 .mu.M pepstatin A, pH 8.0 were added. The cells were homogenized using a glass tissue grinder and teflon pestle. Samples were ultracentrifuged at 100,000.times.g for 1 hr at 0.degree. C. Supernatants were assayed at 0.25 .mu.M cGMP using the method from Thompson, W. J. et. al. Adv. Cyclic Nucleotide Res., 10: 69-92, 1979. Again, the level of cGMP hydrolytic activity increased upon TPA treatment/activation, compared with no treatment/unactivation (see FIG. 7). Both of these experiments corroborate the results of our experiments above that show that both cGMP **PDE2** and **PDE5** protein levels increase in U937 cells treated with TPA.

DETD The activity of specific PDE inhibitors contrast with the activity of compound 38 in U937 cells. By "specific" in this context, we mean the other PDE inhibitors that inhibited one PDE primarily, but not several PDEs (e.g., inhibiting **PDE2** and **PDE5** at roughly the same concentration). An example is sildenafil, which primarily inhibits **PDE5**, and only at much higher concentrations may only marginally inhibit other PDEs. Another example is rolipram (PDE4-specific).

DETD U937 cells were incubated in the presence of 0.3 nM sildenafil or 0.5 uM rolipram for 24 hours using the culture conditions described above. The cells were harvested and processed for IIF as described above using an

antibody that specifically recognizes active caspase 3. Digital images are shown in FIGS. 10 and 11. No increase in the levels of apoptosis compared to normal background was observed. Therefore, the inhibition of only PDE4 or **PDE5** alone (i.e. without the inhibition of **PDE2**) is not sufficient to induce apoptosis in U937 cells.

DETD The BBDP/Wor rat pancreatic tissue specimens were fixed in 10% buffered formalin, paraffin embedded, sectioned at 5 .mu.m and stored at room temperature in a slide box until use. Sections were then dewaxed for 2 hours at 60.degree. C. and deparaffinized in xylene, three incubations, two minutes each. Sections were rehydrated through an ethanol series at three different percentages (100, 95 and 70%), twice each for two minutes, followed by five minutes of rinsing in phosphate buffered saline. Endogenous peroxidase activity was blocked by incubating pancreatic sections in fresh 0.3% hydrogen peroxide at room temperature for 30 minutes. Sections were washed in phosphate buffered saline for 5 minutes, then blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in phosphate buffered saline) for one hour. Tissue sections were incubated with primary antibody diluted in blocking buffer (affinity purified sheep anti-human **PDE2**(1), diluted 1:1500 and affinity purified sheep anti-human **PDE5**(1), diluted 1:2000) and were incubated overnight at 4.degree. C. in a humid chamber. Sections were washed three times for five minutes each in phosphate buffered saline. Sections were incubated with a secondary antibody (diluted donkey anti-sheep biotin conjugated immunoabsorbed antibody 1:2500) in blocking buffer, Jackson lmmunoResearch Catalog #713-065-147. Sections were then incubated at room temperature for 30 minutes, washed three times in phosphate buffered saline for 5 minutes. Diluted Vector ABC reagents were used as according to manufacture's protocol (vector Catalog #6106) and were incubated for 30 minutes at room temperature. Washed in phosphate buffered saline for 5 minutes. A DAB substrate kit was used per manufacture's protocol (Vector Catalog #SK-4100). Slides containing tissue were rinsed with distilled water and immediately immerse in Meyers hematoxylin for 1-5 minutes. Excess hematoxylin was rinsed off with distilled water. Slides were dipped 10 times in 2% glacial acetic acid solution followed by 10 dips in distilled water. Slides were incubated in bluing solution (15 mM ammonium water) 15 dips followed by 10 dips in water and dehydrated through an ethanol series. The labelled slides were then mounted in Permount (Sigma).

DETD Quantitative **PDE2** and **PDE5** Immunohistochemistry

DETD Rat pancreatic tissues on microscope slides were analyzed by the Automated Cellular Imaging System (Chromavision, Capistrano, Calif.) using the generic DAB application (rev. A). Each tissue specimen was thoroughly scanned and 10 islet regions were examined and areas of necrosis were avoided. Percentage of cells with positive signal for **PDE2** and **PDE5** was computed. See table below for generated values (Table I under results section).

DETD With these results, one aspect of this invention is a medical therapy that involves the administration of the **PDE2** inhibitor (preferably also a **PDE5** inhibitor) at the stage of Type I diabetes development when macrophage invasion is occurring. Macrophages release cytokines that can recruit other inflammatory cells such as lymphocytes and neutrophils, all of which can cause damage to islet cells. Thus, it is desirable to begin therapy at the stage of macrophage activation.

DETD

TABLE I

Quantitative Immunohistochemistry of **PDE2** and **PDE5** in Islet Cell Region

Tissue	Mean Number of Cells with Brown Intensity in Islet Cells	Mean Number of Cells with Brown Intensity in Islet Cells

Identification Stage of Disease (**PDE2**) (**PDE5**)

B14A Chronic Type-I 85 .+-. 32.9 111 .+-. 14.5
B14D B14A age-matched 71 .+-. 32.6 87 .+-. 7.2
resistant control
B14B Acute Type-I 56 .+-. 26.5 96 .+-. 6.8
B14E B14B age-matched 78 .+-. 6.3 102 .+-. 8.5
resistant control
B14C Prone Type-I 58 .+-. 33.2 87 .+-. 6.4
B14F B14C age-matched 57 .+-. 39.8 93 .+-. 4.9
resistant control

DETD Human formalin-fixed paraffin-embedded 5-.mu.m thick pancreatic tissue was obtained from a 22 year-old female and another female of unknown age. Both patients had a history of Type-I diabetes mellitus. A serial dilution study demonstrated the optimal signal-to-noise ratio was 1:100 and 1:200 (**PDE2**), 1:500 and 1:1000 (**PDE5**). Anti-**PDE2** and anti-**PDE5** antibodies were used as the primary antibodies, and the principal detection system consisted of a Vector anti-sheep secondary (BA-6000) and Vector ABC-AP Kit (AK-5000) with a Vector Red substrate kit (SK-5100), which was used to produce a fuchsia-colored red deposit. Tissues were also stained with a positive control antibody (CD31) to ensure the tissue antigens were preserved and accessible for immunohistochemical analysis. CD31 is present in monocytes, macrophages, granulocytes, B lymphocytes and platelets. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged using a DVC Digital Photo Camera coupled to a Nikon microscope.

DETD Human pancreatic tissue samples (1 and 2) exhibited positive staining for **PDE2** and **PDE5** proteins and immunostaining was mostly localized to pancreatic islet cells. In FIGS. 17a and 17b, pancreatic tissue specimens from the two human patients described above are stained for **PDE2** protein. In FIGS. 18a and 18b pancreatic tissue specimens from the two human patients described above are stained for **PDE5** protein.

CLM What is claimed is:
3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

34. The method of claim 33 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

PI US 6479493 B1 20021112

L2 ANSWER 42 OF 92 USPATFULL

SUMM This invention represents a novel therapy for treating patients (e.g., humans or companion animals) with type I diabetes without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 2 ("**PDE2**") to a mammal in need of treatment for type I diabetes. Preferably, that inhibitor also inhibits phosphodiesterase 5 ("**PDE5**"). In narrower aspects of this invention, this invention involves the administration of compounds of Formula I below to a mammal in need of treatment for type I diabetes.

DRWD FIG. 1 is a graph that compares the **PDE2** and **PDE5** MRNA levels in control and activated macrophages.

L2 ANSWER 34 OF 92 USPATFULL

SUMM This invention relates to the use of one or more forms of phosphodiesterase type 2 ("**PDE2**") and phosphodiesterase type 5 ("**PDE5**") and/or protein kinase G to identify compounds useful for the treatment and prevention of pre-cancerous and cancerous lesions in mammals, and to pharmaceutical compositions containing such compounds, as well as to therapeutic methods of treating neoplasia with such compounds.

SUMM In the course of researching why some **PDE5** inhibitors singly induced apoptosis while others did not, we uncovered a form of cyclic GMP-specific phosphodiesterase activity, not previously described. This new phosphodiesterase activity was previously uncharacterized. . Without being limited to a specific theory, we believe this novel PDE activity may be a novel conformation of **PDE2** that substantially lacks cAMP-hydrolyzing activity, i.e. it is cGMP-specific. Classic **PDE2** is not cGMP-specific (it also hydrolyzes cAMP), classic **PDE2** is also found in neoplastic cells. This new PDE and **PDE2** are useful in screening pharmaceutical compounds for desirable anti-neoplastic properties. Basically, eoplastic cells when **PDE5** and the **PDE2** activity (in its novel and conventional conformations) are inhibited by an anti-neoplastic **PDE5**-inhibiting compound, the result is apoptosis. When only **PDE5** is inhibited (but not the several forms of **PDE2**), apoptosis does not occur.

SUMM Another embodiment of this invention involves evaluating whether a compound causes an increase in cGMP-dependent protein kinase G ("PKG") activity and/or a decrease of .beta.-catenin in neoplastic cells. It has been found that unexpected characteristics of SAANDs include the elevation of PKG activity and a decrease in .beta.-catenin in neoplastic cells exposed to a SAAND. We believe that the elevation of PKG activity is due at least in part by the increase in cGMP caused by SAANDs inhibition of the appropriate PDEs, as described above. The other characteristics of SAANDs are (1) inhibition of **PDE5** as reported in the '694 Patent above, (2) inhibition of the novel cGMP-specific PDE conformation, (3) inhibition of **PDE2**; (4) the fact that they increase intracellular cGMP in neoplastic cells, and (5) the fact that they decrease cAMP levels in some types of neoplastic cells.

SUMM Thus, one embodiment of the novel method of this invention is evaluating hether a compound causes PKG activity to elevate in neoplastic cells and whether hat compound inhibits **PDE5**. Another embodiment of the novel screening method of his invention is evaluating whether a compound that causes PKG activity to elevate in neoplastic cells and whether that compound inhibits the novel cGMP-specific PDE described above and/or **PDE2**. Still a third embodiment is evaluating whether a compound causes PKG activity to elevate in neoplastic cells and whether that compound causes cGMP to rise in neoplastic cells and/or causes cAMP levels to fall. Compounds successfully evaluated in such fashions have application as SAANDs.

DETD The novel PDE of this invention and **PDE2** are useful with or without **PDE5** to identify compounds that can be used to treat or prevent neoplasms, and that are not characterized by serious side effects.

DETD Obviously, a compound that exhibits a lower COX-I or COX-2 inhibitory activity in relation to its greater combined **PDE5**/novel PDE/ **PDE2** inhibitory activities may be a desirable compound.

DETD Compounds can be screened for inhibitory effect on the activity of the novel phosphodiesterase of this invention using either the enzyme isolated as described above, a recombinant version, or using the novel PDE and/or **PDE2** together with **PDE5**. Alternatively,

cyclic nucleotide levels in whole cells are measured by RIA and compared to untreated and zaprinast-treated cells.

PI US 6500610 B1 20021231

A1 20030116

L2 ANSWER 31 OF 92 USPATFULL

SUMM [0028] A "selective PDE10 inhibitor" can be identified, for example, by comparing the ability of a substance to inhibit PDE10 activity to its ability to inhibit PDE enzymes from the other PDE families. For example, a substance may be assayed for its ability to inhibit PDE10 activity, as well as PDE1, **PDE2**, PDE3A, PDE4A, PDE4B, PDE4C, PDE4D, **PDE5**, PDE6, PDE7, PDE8, PDE9, and PDE11.

DETD [0047] We observed that papaverine was an exceptionally potent, competitive inhibitor of PDE10 with an IC.sub.50 value of 18 nM (Table 1). Papaverine was considerably less potent against all other PDEs tested. After PDE10, the enzyme inhibited most potently by papaverine was PDE4D with an IC.sub.50 of 320 nM, a value 19-fold lower than that for PDE10. Thus, these data reveal for the first time that papaverine is a selective PDE10 inhibitor and that this compound can be used in studies of this enzyme's physiology.

TABLE 1

IC.sub.50 values for papaverine inhibition of the listed PDEs. IC.sub.50s were determined for each enzyme at a substrate concentration of 1/3 the Km value to allow for comparisons across enzymes. The PDE10 selectivity ratio is the IC.sub.50 value for a given PDE divided by the IC.sub.50 value for PDE10.

Isozyme	IC.sub.50, .mu.M	Selectivity Ratio (IC.sub.50/IC.sub.50, PDE10)
PDE10	0.018	--
PDE1	37	2,055
PDE2	9	500
PDE3A	1.3	72
PDE4A	1.9	105
PDE4B	1.4	78
PDE4C	0.8	44
PDE4D	0.32	18
PDE5	8	444
PDE6	0.86	48
PDE7	27	1,500
PDE8	>10	>555
PDE9	400	20,000
PDE11	11	611

PI US 2003008806 A1 20030109

L2 ANSWER 32 OF 92 USPATFULL

SUMM The required PDE enzymes were isolated from a variety of sources, including human corpus cavernosum, human and rabbit platelets, human cardiac ventricle, human skeletal muscle and bovine retina, essentially by the method of W. J. Thompson and M. M. Appleman (Biochem., 1971, 10, 311). In particular, the cGMP-specific PDE (**PDE5**) and the cGMP-inhibited cAMP PDE (PDE3) were obtained from human corpus cavernosum tissue, human platelets or rabbit platelets; the cGMP-stimulated PDE (**PDE2**) was obtained from human corpus cavernosum; the calcium/calmodulin (Ca/CAM)-dependent PDE (PDE1) from human cardiac ventricle; the cAMP-specific PDE (PDE4) from human skeletal muscle; and the photoreceptor PDE (PDE6) from bovine retina. Phosphodiesterases 7-11 were generated from full length human recombinant clones transfected into SF9 cells.

PI US 6503908 B1 20030107

L2 ANSWER 21 OF 92 USPATFULL

SUMM [0385] The required PDE enzymes were isolated from a variety of sources, including human corpus cavernosum, human platelets, human cardiac ventricle, human skeletal muscle and human and canine retina, essentially by the method of W. J. Thompson and M. M. Appleman (Biochem., 1971, 10, 311). In particular, the cGMP-specific PDE (**PDE5**) and the cGMP-inhibited cAMP PDE (PDE3) were obtained from human corpus cavernosum tissue or human platelets; the cGMP-stimulated PDE (**PDE2**) was obtained from human corpus cavernosum or human platelets; the calcium/calmodulin (Ca/CAM)-dependent PDE (PDE1) from human cardiac ventricle; the cAMP-specific PDE (PDE4) from recombinant clone or human skeletal muscle; and the photoreceptor PDE (PDE6) from canine or human retina. Phosphodiesterases 7-11 were generated from full length human recombinant clones transfected into SF9 cells.

PI US 2003064990 A1 20030403

L2 ANSWER 22 OF 92 USPATFULL

SUMM [0156] viii) PDE inhibitors such as **PDE2** (e.g. erythro-9-(2-hydroxyl-3-nonyl)-adenine) and Example 100 of EP 0771799-incorporated herein by reference) and in particular a **PDE5** inhibitor (e.g. sildenafil, 1-{[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-trazin-2-yl)-4-ethoxyphenyl]sulfonyl}-4-ethylpiperazine i.e. vardenafil/Bayer BA 38-9456 or IC351 (see structure below, lcos Lilly)). A possible rationale for PDE inhibitors treating premature ejaculation is as follows. c AMP and CGMP levels in the ejaculatory smooth muscles regulate muscle tone of these ejaculatory muscles and so delay ejaculation. ##STR11##

PI US 2003060456 A1 20030327

L2 ANSWER 23 OF 92 USPATFULL

SUMM This invention represents a novel therapy for treating renal cell carcinoma patients without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 10 ("PDE10"). Such an inhibitor also advantageously inhibits **PDE2** and **PDE5**.

DETD Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998, now abandoned. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, asolution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400.mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter.

The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for **PDE5** of 4 .mu.M, an IC.sub.50 value for PDEI of 3 .mu.M, and an IC.sub.50 value for PDE4 of 6 .mu.M.

DETD To identify new compounds that inhibit PDE10 in the manner of this invention, one can retrace what we have taught in this application. Namely, a compound according to this invention can be found by evaluating its ability to inhibit PDE10 as taught above. Alternatively, the compound can be identified by its ability to inhibit cGMP PDEs non-selectively, (i.e., at least inhibit PDE10 and **PDE2** and **PDE5**. It is also believed to be desirable to inhibit PDE1). As confirmation that a desired compound has been identified, one can then assess whether PDE10 is inhibited, and as further confirmation the compound causes cell death, preferably by apoptosis. These procedures are described above, and individually, but not in combination, known in the art.

CLM What is claimed is:

6. The method of claim 5 wherein said inhibitor of PDE10, **PDE2** and **PDE5** comprise the same compound.

PI US 6538029 B1 20030325

L2 ANSWER 20 OF 92 USPATFULL

SUMM [0010] This invention represents a novel therapy for treating patients (e.g., humans or companion animals) with scleroderma without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 2 ("**PDE2**") to a mammal in need of treatment for scleroderma. Preferably, that inhibitor also inhibits phosphodiesterase 5 ("**PDE5**"). In narrower aspects of this invention, this invention involves the administration of compounds of Formula I below to a mammal in need of treatment for scleroderma.

DRWD [0013] FIG. 1 is a graph that compares the **PDE2** and **PDE5** MRNA levels in control and activated macrophages.

DETD [0025] As discussed above, the present invention includes the administration of an inhibitor of **PDE2** to a mammal in need of treatment for scleroderma. Preferably, the compound also inhibits **PDE5**. In addition, this invention includes the use of compounds of Formula I below (as well as their pharmaceutically acceptable salts) for treating a mammal with scleroderma: ##STR1##

DETD [0043] Compounds of this invention are inhibitors of phosphodiesterases **PDE2**. For convenience, the PDE inhibitory activity of such compounds can be tested as taught in U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998 to Pamukcu et al., which is incorporated herein by reference. Thus, compounds employed in this invention are useful inhibitors of **PDE2** and preferably also **PDE5**. Most preferably, such compounds have an IC.sub.50 for **PDE2** of no more than 25 .mu.M.

DETD [0044] Additional compounds besides those of Formula I can be identified for inhibitory effect on the activity of **PDE2** and/or **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by radioimmunoassay ("RIA") and compared to untreated and drug-treated tissue samples and/or isolated enzymes.

DETD [0045] Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for the PDE enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/mL BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with isolated **PDE2** and/or **PDE5**. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/mL snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 minutes at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 mL of 100% methanol. Assay samples are applied to 1 mL Dowex 1-X8 column; and washed with 1 mL of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the column is combined and measured with a scintillation counter. The degree of phosphodiesterase inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound but with drug solvent).

DETD [0049] In addition to compounds disclosed herein, other compounds that inhibit both **PDE2** and **PDE5** include compounds disclosed in U.S. Pat. Nos. 5,401,774 (e.g., exisulind), 6,063,818, 5,998,477, and 5,965,619. These patents are incorporated herein by reference. Preferable compounds include those having a **PDE2** IC.sub.50 less than about 25 .mu.M.

DETD [0050] When referring to an "a physiologically effective amount of an inhibitor of **PDE2** and **PDE5**" we mean not only a single compound that inhibits those enzymes but a combination of several compounds, each of which can inhibit one or both of those enzymes.

Single compounds that inhibit both enzymes are preferred.

DETD [0051] When referring to an "inhibitor [that] does not substantially inhibit COX I or COX II," we mean that in the ordinary sense of the term. By way of example only, if the inhibitor has an IC.sub.50 for either **PDE2** or **PDE5** that is at least half of the IC.sub.50 of COXI and/or COXII, a drug achieving the PDE IC.sub.50 in the blood could be said not to substantially inhibit the COX enzymes. Preferably, the IC.sub.50 for the COX enzymes is in the order of 10 fold or more higher than the IC.sub.50 for **PDE2**/**PDE5**. Preferably the IC.sub.50 for each of the COX enzymes is greater than about 40 .mu.M.

DETD [0219] As to identifying structurally additional **PDE2** and **PDE5** inhibiting compounds besides those of Formula I that can be effective therapeutically for SCLERODERMA, one skilled in the art has a number of useful model compounds disclosed herein (as well as their analogs) that can be used as the bases for computer modeling of additional compounds having the same conformations but different chemically. For example, software such as that sold by Molecular Simulations Inc. release of WebLab.RTM. ViewerPro.TM. includes molecular visualization and chemical communication capabilities. Such software includes functionality, including 3D visualization of known active compounds to validate sketched or imported chemical structures for accuracy. In addition, the software allows structures to be superimposed based on user-defined features, and the user can measure distances, angles, or dihedrals.

DETD [0222] To further assist in identifying compounds that can be screened and then selected using the criterion of this invention, knowing the binding of selected compounds to **PDE5** and **PDE2** protein is of interest. By the procedures discussed below, it is believed that that preferable, desirable compounds meeting the selection criteria of this invention bind to the cGMP catalytic regions of **PDE2** and **PDE5**.

DETD [0235] Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400.mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter. The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD [0236] Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for **PDE5** of 4 .mu.M, an IC.sub.50 value for PDE1 of 3 .mu.M, and an

IC.sub.50 value for PDE4 of 6 .mu.M.

DETD [0249] As demonstrated below, we found that macrophages contain **PDE2** and **PDE5**, and the inhibition of **PDE2** particularly with **PDE5** inhibition leads to apoptosis of macrophage cells. We believe the administration of a **PDE2** inhibitor can treat the progression of scleroderma, particularly when **PDE5** is also inhibited.

DETD ii. **PDE2** and **PDE5** mRNA Levels in Treated and Untreated U937 Cells by RT-PCR

DETD [0251] U937 cells (from ATCC Rockville, Md.) were grown in RPMI media supplemented with 5% FCS, glutamine, antibiotic/antimycotic and sodium pyruvate. Total RNA was isolated from two U937 cultures, one treated with 5 nM TPA for 48 hours and one grown in normal media as listed above, using the Rouche High Pure RNA Isolation Kit (cat# 1 828 665) as per manufacturers protocol. cDNA was then synthesized from the total RNA using GibcoBRL SuperscriptII (Cat # 18064-022) reverse transcriptase as per manufacturers protocol. The resulting cDNA was used as a template for RT-PCR reactions using primer sets specific for **PDE2** (forward: CCCAAAGTGGAGACTGTCTACACCTAC, reverse: CCGGTTGTCTTCCAGCGTGTC) or **PDE5** (forward: GGGACTTTACCTTCTCATAC, reverse. GTGACATCCAAATGACTAGA). mRNA for **PDE2** and 5 were both present in the untreated U937 cells. Upon treatment with TPA, the relative amounts of **PDE2** mRNA increased 5 fold. Therefore, U937 cells treated with TPA and driven to differentiate into an activated macrophage like state have elevated levels of **PDE2** mRNA (see FIG. 1).

DETD iii. Confirmation of **PDE2** and **PDE5** Protein Within U937 Cells by Indirect Immunofluorescence

DETD [0252] The presence of **PDE2** and **PDE5** protein within U937 cells was confirmed by indirect immunofluorescence (IIF). U937 cells were cultured as above. Two U937 cultures, one grown in the presence of 5nM TPA for 48 hours and one grown in normal media were processed. All cultures were collected by centrifugation (Shandon Cytospin, 2 minutes @ 600 rpm) onto poly-L lysine-coated slides and immediately fixed in fresh 3% paraformaldehyde buffered in PBS for 10 minutes. Adherent cultures were grown on coverslips and fixed as above. Cells were permeablized in 0.2% triton-100 for 2 minutes. Slides were blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in PBS) for 1 hour at room temperature.

DETD [0253] Slides were then incubated for 1 hour at 37.degree. C. in a humid chamber with antibodies specific for **PDE2** (generated in a sheep against the peptide TLAFQKEQKLKCECQA) or **PDE5** (generated in sheep against the peptide CAQLYETSLLENKRNQV). The **PDE5** antibody was used at a dilution of 1:200 and the **PDE2** antibody was used at a dilution of 1:100. All dilutions were performed in blocking buffer. Slides were then washed 2.times. for 10 minutes each in PBS and then incubated with a Cy3 conjugated secondary antibody (Jackson ImmunoResearch laboratories, Inc. Cat. # 713-166-147) diluted 1:1000 in blocking buffer, for 1 hour at 37.degree. C. in a humid chamber. Slides were then washed 2.times. for 10 minutes each in PBS and counterstained with DAPI (5 ng/ml) and mounted in VectaShield. Digital images were then obtained using a SPOT-2 camera and an Olympus IX-70 fluorescent microscope. Both **PDE2** and **PDE5** are present in the cytoplasm of U937 cells. There is an increase in the level of both **PDE2** and **PDE5** in TPA-treated U937 cells. These increased protein levels are seen in discrete perinuclear foci (see FIGS. 2 through 5).

DETD [0256] cGMP hydrolysis levels in protein lysates extracted from TPA-treated and untreated U937 cells were also analyzed as follows. Cells were resuspended in 20 mM TRIS-HCl, 5 mM MgCl2, 0.5% Triton X-100, 0.1 mM EDTA, 10 mM benzamidine, 10 .mu.M TLCK, 20 nM aprotinin, 2 .mu.M leupeptin, 2 .mu.M pepstatin A, pH 8.0 were added. The cells were homogenized using a glass tissue grinder and teflon pestle. Samples were

ultracentrifuged at 100,000.times.g for 1 hr at 0.degree. C. Supernatants were assayed at 0.25 .mu.M cGMP using the method from Thompson, W. J. et. al. Adv. Cyclic Nucleotide Res., 10: 69-92, 1979. Again, the level of cGMP hydrolytic activity increased upon TPA treatment/activation, compared with no treatment/unactivation (see FIG. 7). Both of these experiments corroborate the results of our experiments above that show that both cGMP **PDE2** and **PDE5** protein levels increase in U937 cells treated with TPA.

DETD [0260] The activity of specific PDE inhibitors contrast with the activity of compound 38 in U937 cells. By "specific" in this context, we mean the other PDE inhibitors that inhibited one PDE primarily, but not several PDEs (e.g., inhibiting **PDE2** and **PDE5** at roughly the same concentration). An example is sildenafil, which primarily inhibits **PDE5**, and only at much higher concentrations may only marginally inhibit other PDEs. Another example is rolipram (PDE4-specific).

DETD [0261] U937 cells were incubated in the presence of 0.3nM sildenafil or 0.5uM rolipram for 24 hours using the culture conditions described above. The cells were harvested and processed for IIF as described above using an antibody that specifically recognizes active caspase 3. Digital images are shown in FIGS. 10 and 11. No increase in the levels of apoptosis compared to normal background was observed. Therefore, the inhibition of only PDE4 or **PDE5** alone (i.e. without the inhibition of **PDE2**) is not sufficient to induce apoptosis in U937 cells.

CLM What is claimed is:

3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

33. The method of claim 32 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

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SUMM [0018] This invention represents a novel therapy for treating patients (e.g., humans or companion animals) with lupus erythematosus without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 2 ("**PDE2**") to a mammal in need of treatment for lupus erythematosus. Preferably, that inhibitor also inhibits phosphodiesterase 5 ("**PDE5**"). In narrower aspects of this invention, this invention involves the administration of compounds of Formula I below to a mammal in need of treatment for lupus erythematosus.

DRWD [0021] FIG. 1 is a graph that compares the **PDE2** and **PDE5** mRNA levels in control and activated macrophages.

DETD [0055] Compounds of this invention are inhibitors of phosphodiesterases **PDE2**. For convenience, the PDE inhibitory activity of such compounds can be tested as taught in U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998 to Pamukcu et al., which is incorporated herein by reference. Thus, compounds employed in this invention are useful inhibitors of **PDE2** and preferably also **PDE5**. Most preferably, such compounds have an IC.sub.50 for **PDE2** of no more than 25 .mu.M.

DETD [0056] Additional compounds besides those of Formula I can be identified for inhibitory effect on the activity of **PDE2** and/or **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by radioimmunoassay ("RIA") and compared to untreated and drug-treated tissue samples and/or isolated enzymes.

DETD [0057] Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for the PDE enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/mL BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with isolated **PDE2** and/or **PDE5**. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 pi of 0.5 mg/mL snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 minutes at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 mL of 100% methanol. Assay samples are applied to 1 mL Dowex 1-X8 column; and washed with 1 mL of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the column is combined and measured with a scintillation counter. The degree of phosphodiesterase inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound but with drug solvent).

DETD [0061] In addition to compounds disclosed herein, other compounds that inhibit both **PDE2** and **PDE5** include compounds disclosed in U.S. Pat. Nos. 5,401,774 (e.g., exisulind), 6,063,818, 5,998,477, and 5,965,619. These patents are incorporated herein by reference.

DETD [0062] When referring to an "a physiologically effective amount of an inhibitor of **PDE2** and **PDE5**" we mean not only a single compound that inhibits those enzymes but a combination of several compounds, each of which can inhibit one or both of those enzymes. Single compounds that inhibit both enzymes are preferred.

DETD [0063] When referring to an "inhibitor [that] does not substantially inhibit COX I or COX II," we mean that in the ordinary sense of the term. By way of example only, if the inhibitor has an IC.sub.50 for either **PDE2** or **PDE5** that is at least half of the

IC.sub.50 of COXI and/or COXII, a drug achieving the PDE IC.sub.50 in the blood could be said not to substantially inhibit the COX enzymes. Preferably, the IC.sub.50 for the COX enzymes is in the order of 10 fold or more higher than the IC.sub.50 for **PDE2/PDE5**.
Preferably, the IC.sub.50 for the COX enzymes is greater than about 40 .mu.M.

DETD [0266] As to identifying structurally additional **PDE2** and **PDE5** inhibiting compounds besides those of Formula I that can be effective therapeutically for lupus erythematosus, one skilled in the art has a number of useful model compounds disclosed herein (as well as their analogs) that can be used as the bases for computer modeling of additional compounds having the same conformations but different chemically. For example, software such as that sold by Molecular Simulations Inc. release of WebLab.RTM. ViewerPro.TM. includes molecular visualization and chemical communication capabilities. Such software includes functionality, including 3D visualization of known active compounds to validate sketched or imported chemical structures for accuracy. In addition, the software allows structures to be superimposed based on user-defined features, and the user can measure distances, angles, or dihedrals.

DETD [0269] To further assist in identifying compounds that can be screened and then selected using the criterion of this invention, knowing the binding of selected compounds to **PDE5** and **PDE2** protein is of interest. By the procedures discussed below, it is believed that that preferable, desirable compounds meeting the selection criteria of this invention bind to the cGMP catalytic regions of **PDE2** and **PDE5**.

DETD [0277] Examples of compounds that inhibit **PDE2** and **PDE5** (with insubstantial COX inhibition) include exisulind and compounds disclosed in U.S. Pat. Nos. 5,965,619 and 6,063,818 which are incorporated herein by reference.

DETD [0283] Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter. The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD [0284] Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for **PDE5** of 4 .mu.M, an IC.sub.50 value for PDE1 of 3 .mu.M, and an IC.sub.50 value for PDE4 of 6 .mu.M.

DETD [0298] As demonstrated below, we found that macrophages contain **PDE2** and **PDE5**, and the inhibition of **PDE2** particularly with **PDE5** inhibition leads to apoptosis of macrophage cells. We believe the administration of a **PDE2** inhibitor can treat the progression of lupus erythematosus, particularly when **PDE5** is also inhibited.

DETD [0299] ii. **PDE2** and **PDE5** mRNA Levels in Treated and Untreated U937 Cells by RT-PCR

DETD [0301] U937 cells (from ATCC Rockville, Md.) were grown in RPMI media supplemented with 5% FCS, glutamine, antibiotic/antimycotic and sodium pyruvate. Total RNA was isolated from two U937 cultures, one treated with 5 nM TPA for 48 hours and one grown in normal media as listed above, using the Rouche High Pure RNA Isolation Kit (cat# 1 828 665) as per manufacturers protocol. cDNA was then synthesized from the total RNA using GibcoBRL SuperscriptII (Cat # 18064-022) reverse transcriptase as per manufacturers protocol. The resulting cDNA was used as a template for RT-PCR reactions using primer sets specific for **PDE2** (forward: CCCAAAGTGGAGACTGTCTACACCTAC, reverse: CCGGTTGTCTTCCAGCGTGTC) or **PDE5** (forward: GGGACTTTACCTTCTCATAC, reverse: GTGACATCCAAATGACTAGA). mRNA for **PDE2** and 5 were both present in the untreated U937 cells. Upon treatment with TPA, the relative amounts of **PDE2** mRNA increased 5 fold. Therefore, U937 cells treated with TPA and driven to differentiate into an activated macrophage like state have elevated levels of **PDE2** mRNA (see FIG. 1).

DETD [0302] iii. Confirmation of **PDE2** and **PDE5** Protein Within U937 Cells by Indirect Immunofluorescence

DETD [0303] The presence of **PDE2** and **PDE5** protein within U937 cells was confirmed by indirect immunofluorescence (IIF). U937 cells were cultured as above. Two U937 cultures, one grown in the presence of 5 nM TPA for 48 hours and one grown in normal media were processed. All cultures were collected by centrifugation (Shandon Cytospin, 2 minutes @ 600 rpm) onto poly-L lysine-coated slides and immediately fixed in fresh 3% paraformaldehyde buffered in PBS for 10 minutes. Adherent cultures were grown on coverslips and fixed as above. Cells were permeablized in 0.2% triton-100 for 2 minutes. Slides were blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in PBS) for 1 hour at room temperature.

DETD [0304] Slides were then incubated for 1 hour at 37.degree. C. in a humid chamber with antibodies specific for **PDE2** (generated in a sheep against the peptide TLAFQKEQKLKCECQA) or **PDE5** (generated in sheep against the peptide CAQLYETSLLENKRNQV). The **PDE5** antibody was used at a dilution of 1:200 and the **PDE2** antibody was used at a dilution of 1:100. All dilutions were performed in blocking buffer. Slides were then washed 2.times. for 10 minutes each in PBS and then incubated with a Cy3 conjugated secondary antibody (Jackson ImmunoResearch laboratories, Inc. Cat. # 713-166-147) diluted 1:1000 in blocking buffer, for 1 hour at 37.degree. C. in a humid chamber. Slides were then washed 2x for 10 minutes each in PBS and counterstained with DAPI (5 ng/ml) and mounted in VectaShield. Digital images were then obtained using a SPOT-2 camera and an Olympus IX-70 fluorescent microscope. Both **PDE2** and **PDE5** are present in the cytoplasm of U937 cells. There is an increase in the level of both **PDE2** and **PDE5** in TPA-treated U937 cells. These increased protein levels are seen in discrete perinuclear foci (see FIGS. 2 through 5).

DETD [0308] cGMP hydrolysis levels in protein lysates extracted from TPA-treated and untreated U937 cells were also analyzed as follows. Cells were resuspended in 20 mM TRIS-HCl, 5 mM MgCl2, 0.5% Triton X-100, 0.1 mM EDTA, 10 mM benzamidine, 10 .mu.M TLCK, 20 nM aprotinin, 2 .mu.M leupeptin, 2 .mu.M pepstatin A, pH 8.0 were added. The cells were homogenized using a glass tissue grinder and teflon pestle. Samples were ultracentrifuged at 100,000.times.g for 1 hr at 0.degree. C.

Supernatants were assayed at 0.25 .mu.M cGMP using the method from Thompson, W. J. et. al. Adv. Cyclic Nucleotide Res., 10: 69-92, 1979. Again, the level of cGMP hydrolytic activity increased upon TPA treatment/activation, compared with no treatment/unactivation (see FIG. 7). Both of these experiments corroborate the results of our experiments above that show that both cGMP **PDE2** and **PDE5** protein levels increase in U937 cells treated with TPA.

DETD [0314] The activity of specific PDE inhibitors contrast with the activity of compound 38 in U937 cells. By "specific" in this context, we mean the other PDE inhibitors that inhibited one PDE primarily, but not several PDEs (e.g., inhibiting **PDE2** and **PDE5** at roughly the same concentration). An example is sildenafil, which primarily inhibits **PDE5**, and only at much higher concentrations may only marginally inhibit other PDEs. Another example is rolipram (PDE4-specific).

DETD [0315] U937 cells were incubated in the presence of 0.3 nM sildenafil or 0.5 uM rolipram for 24 hours using the culture conditions described above. The cells were harvested and processed for IIF as described above using an antibody that specifically recognizes active caspase 3. Digital images are shown in FIGS. 10 and 11. No increase in the levels of apoptosis compared to normal background was observed. Therefore, the inhibition of only PDE4 or **PDE5** alone (i.e. without the inhibition of **PDE2**) is not sufficient to induce apoptosis in U937 cells.

DETD [0320] Skin tissue was obtained from a patient with a known history of discoid lupus erythematosus. Tissue was formalin-fixed, processed and sections at a thickness of 5 .mu.m. A serial dilution study demonstrated the optimal signal-to-noise ratio was 1:100 and 1:200 (**PDE2**), 1:500 and 1:1000 (**PDE5**). Anti-**PDE2** and anti-**PDE5** was used as the primary antibodies, and the principal detection system consisted of a Vector anti-sheep secondary (BA-6000) and Vector ABC-AP Kit (AK-5000) with a Vector Red substrate kit (SK-5100), which was used to produce a fuchsia-colored red deposit. Tissues were also stained with a positive control antibody (CD31) to ensure the tissue antigens were preserved and accessible for immunohistochemical analysis. CD31 is present in monocytes, macrophages, granulocytes, B lymphocytes and platelets. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged using a DVC Digital Photo Camera coupled to a Nikon microscope.

DETD [0321] As shown in FIGS. 13-15, human skin discoid lupus erythematosus tissue samples exhibited positive staining for **PDE2** and **PDE5** immunoactivity that was mostly localized to macrophages, lymphocytes and neutrophils. FIGS. 13 and 14 are visual images of immunostaining to **PDE2** protein and FIG. 15 is a visual image of immunostaining to **PDE5** protein.

CLM What is claimed is:

3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

33. The method of claim 32 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

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SUMM [0011] This invention represents a novel therapy for treating patients (e.g., humans or companion animals) with multiple sclerosis without the substantial side effects of prior pharmaceutical approaches. Specifically, this invention involves the administration of an inhibitor of phosphodiesterase 2 ("**PDE2**") to a mammal in need of treatment for multiple sclerosis. Preferably, that inhibitor also inhibits phosphodiesterase 5 ("**PDE5**"). In narrower aspects of this invention, this invention involves the administration of compounds of Formula I below to a mammal in need of treatment for multiple sclerosis.

DRWD [0014] FIG. 1 is a graph that compares the **PDE2** and **PDE5** mRNA levels in control and activated macrophages.

DETD [0030] As discussed above, the present invention includes the administration of an inhibitor of **PDE2** to a mammal in need of treatment for multiple sclerosis. Preferably, the compound also inhibits **PDE5**. In addition, this invention includes the use of compounds of Formula I below (as well as their pharmaceutically acceptable salts) for treating a mammal with multiple sclerosis: ##STR1##

DETD [0049] Compounds of this invention are inhibitors of phosphodiesterases **PDE2**. For convenience, the PDE inhibitory activity of such compounds can be tested as taught in U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998 to Pamukcu et al., which is incorporated herein by reference. Thus, compounds employed in this invention are useful inhibitors of **PDE2** and preferably also **PDE5**. Most preferably, such compounds have an IC.sub.50 for **PDE2** of no more than 25 .mu.M.

DETD [0050] Additional compounds besides those of Formula I can be identified for inhibitory effect on the activity of **PDE2** and/or **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by radioimmunoassay ("RIA") and compared to untreated and drug-treated tissue samples and/or isolated enzymes.

DETD [0051] Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive 3H cyclic GMP (cGMP)(cyclic 3', 5'-guanosine monophosphate) as the substrate for the PDE enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/mL BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with isolated **PDE2** and/or **PDE5**. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/mL snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 minutes at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 mL of 100% methanol. Assay samples are applied to 1 mL Dowex 1-X8 column; and washed with 1 mL of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the column is combined and measured with a scintillation counter. The degree of phosphodiesterase inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound but with drug solvent).

DETD [0055] In addition to compounds disclosed herein, other compounds that inhibit both **PDE2** and **PDE5** include compounds disclosed in U.S. Pat. Nos. 5,401,774 (e.g., exisulind), 6,063,818, 5,998,477, and 5,965,619. These patents are incorporated herein by reference. Preferable compounds include those having a **PDE2** IC.sub.50 less than about 25 .mu.M.

DETD [0056] When referring to an "a physiologically effective amount of an inhibitor of **PDE2** and **PDE5**" we mean not only a single compound that inhibits those enzymes but a combination of several

compounds, each of which can inhibit one or both of those enzymes. Single compounds that inhibit both enzymes are preferred.

DETD [0057] When referring to an "inhibitor [that] does not substantially inhibit COX I or COX II," we mean that in the ordinary sense of the term. By way of example only, if the inhibitor has an IC.sub.50 for either **PDE2** or **PDE5** that is at least half of the IC.sub.50 of COXI and/or COXII, a drug achieving the PDE IC.sub.50 in the blood could be said not to substantially inhibit the COX enzymes. Preferably, the IC.sub.50 for the COX enzymes is in the order of 10 fold or more higher than the IC.sub.50 for **PDE2**/**PDE5**. Preferably the IC.sub.50 of the compound for each of the COX enzymes is greater than about 40 .mu.M.

DETD [0225] As to identifying structurally additional **PDE2** and **PDE5** inhibiting compounds besides those of Formula I that can be effective therapeutically for MULTIPLE SCLEROSIS, one skilled in the art has a number of useful model compounds disclosed herein (as well as their analogs) that can be used as the bases for computer modeling of additional compounds having the same conformations but different chemically. For example, software such as that sold by Molecular Simulations Inc. release of WebLab.RTM. ViewerPro.TM. includes molecular visualization and chemical communication capabilities. Such software includes functionality, including 3D visualization of known active compounds to validate sketched or imported chemical structures for accuracy. In addition, the software allows structures to be superimposed based on user-defined features, and the user can measure distances, angles, or dihedrals.

DETD [0228] To further assist in identifying compounds that can be screened and then selected using the criterion of this invention, knowing the binding of selected compounds to **PDE5** and **PDE2** protein is of interest. By the procedures discussed below, it is believed that that preferable, desirable compounds meeting the selection criteria of this invention bind to the cGMP catalytic regions of **PDE2** and **PDE5**.

DETD [0241] Compounds of this invention are also **PDE2** and **PDE5** inhibitors as taught in part U.S. patent application Ser. No. 09/046,739 filed Mar. 24, 1998. Compounds can be tested for inhibitory effect on phosphodiesterase activity using either the enzyme isolated from any tumor cell line such as HT-29 or SW-480. Phosphodiesterase activity can be determined using methods known in the art, such as a method using radioactive .sup.3H cyclic GMP (cGMP)(cyclic 3',5'-guanosine monophosphate) as the substrate for **PDE5** enzyme. (Thompson, W. J., Teraski, W. L., Epstein, P. M., Strada, S. J., Advances in Cyclic Nucleotide Research, 10:69-92, 1979, which is incorporated herein by reference). In brief, a solution of defined substrate .sup.3H-cGMP specific activity (0.2 .mu.M; 100,000 cpm; containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl.sub.2 and 1 mg/ml BSA) is mixed with the drug to be tested in a total volume of 400 .mu.l. The mixture is incubated at 30.degree. C. for 10 minutes with partially purified cGMP-specific PDE isolated from HT-29 cells. Reactions are terminated, for example, by boiling the reaction mixture for 75 seconds. After cooling on ice, 100 .mu.l of 0.5 mg/ml snake venom (O. Hannah venom available from Sigma) is added and incubated for 10 min at 30.degree. C. This reaction is then terminated by the addition of an alcohol, e.g. 1 ml of 100% methanol. Assay samples are applied to a anion chromatography column (1 ml Dowex, from Aldrich) and washed with 1 ml of 100% methanol. The amount of radioactivity in the breakthrough and the wash from the columns in then measured with a scintillation counter. The degree of **PDE5** inhibition is determined by calculating the amount of radioactivity in drug-treated reactions and comparing against a control sample (a reaction mixture lacking the tested compound).

DETD [0242] Using such protocols, the compound of Example 1 had an IC.sub.50 value for **PDE5** inhibition of 0.68 .mu.M. Using similar protocols, the compound of Example 38 ("Compound 38") had an IC.sub.50 value for **PDE2** of 14 .mu.M, an IC.sub.50 value for

PDE5 of 4 .mu.M, an IC.sub.50 value for PDE1 of 3 .mu.M, and an IC.sub.50 value for PDE4 of 6 .mu.M.

DETD [0256] As demonstrated below, we found that macrophages contain **PDE2** and **PDE5**, and the inhibition of **PDE2** particularly with **PDE5** inhibition leads to apoptosis of macrophage cells. We believe the administration of a **PDE2** inhibitor can treat the progression of multiple sclerosis, particularly when **PDE5** is also inhibited.

DETD [0257] ii. **PDE2** and **PDE5** mRNA Levels in Treated and Untreated U937 Cells by RT-PCR

DETD [0259] U937 cells (from ATCC Rockville, MD) were grown in RPMI media supplemented with 5% FCS, glutamine, antibiotic/antimycotic and sodium pyruvate. Total RNA was isolated from two U937 cultures, one treated with 5 nM TPA for 48 hours and one grown in normal media as listed above, using the Rouche High Pure RNA Isolation Kit (cat# 1 828 665) as per manufacturers protocol. cDNA was then synthesized from the total RNA using GibcoBRL SuperscriptII (Cat # 18064-022) reverse transcriptase as per manufacturers protocol. The resulting cDNA was used as a template for RT-PCR reactions using primer sets specific for **PDE2** (forward: CCCAAAGTGGAGACTGTCTACACCTAC, reverse: CCGGTTGTCTTCCAGCGTGTC) or **PDE5** (forward: GGGACTTTACCTTCTCATAC, reverse: GTGACATCCAAATGACTAGA). mRNA for **PDE2** and 5 were both present in the untreated U937 cells. Upon treatment with TPA, the relative amounts of **PDE2** mRNA increased 5 fold. Therefore, U937 cells treated with TPA and driven to differentiate into an activated macrophage like state have elevated levels of **PDE2** mRNA (see FIG. 1).

DETD [0260] iii. Confirmation of **PDE2** and **PDE5** Protein Within U937 Cells by Indirect Immunofluorescence

DETD [0261] The presence of **PDE2** and **PDE5** protein within U937 cells was confirmed by indirect immunofluorescence (IIF). U937 cells were cultured as above. Two U937 cultures, one grown in the presence of 5 nM TPA for 48 hours and one grown in normal media were processed. All cultures were collected by centrifugation (Shandon Cytospin, 2 minutes@600 rpm) onto poly-L lysine-coated slides and immediately fixed in fresh 3% paraformaldehyde buffered in PBS for 10 minutes. Adherent cultures were grown on coverslips and fixed as above. Cells were permeablized in 0.2% triton-100 for 2 minutes. Slides were blocked with blocking buffer (5% goat serum, 5% glycerol, 1% gelatin from cold water fish skin and 0.04% NaN.sub.3 in PBS) for 1 hour at room temperature.

DETD [0262] Slides were then incubated for 1 hour at 37.degree. C. in a humid chamber with antibodies specific for **PDE2** (generated in a sheep against the peptide TLAFQKEQKLKCECQA) or **PDE5** (generated in sheep against the peptide CAQLYETSLLENKRNQV). The **PDE5** antibody was used at a dilution of 1:200 and the **PDE2** antibody was used at a dilution of 1:100. All dilutions were performed in blocking buffer. Slides were then washed 2.times. for 10 minutes each in PBS and then incubated with a Cy3 conjugated secondary antibody (Jackson ImmunoResearch laboratories, Inc. Cat. # 713-166-147) diluted 1:1000 in blocking buffer, for 1 hour at 37.degree. C. in a humid chamber. Slides were then washed 2.times. for 10 minutes each in PBS and counterstained with DAPI (5 ng/ml) and mounted in VectaShield. Digital images were then obtained using a SPOT-2 camera and an Olympus IX-70 fluorescent microscope. Both **PDE2** and **PDE5** are present in the cytoplasm of U937 cells. There is an increase in the level of both **PDE2** and **PDE5** in TPA-treated U937 cells. These increased protein levels are seen in discrete perinuclear foci (see FIGS. 2 through 5).

DETD [0266] cGMP hydrolysis levels in protein lysates extracted from TPA-treated and untreated U937 cells were also analyzed as follows. Cells were resuspended in 20 mM TRIS-HCl, 5 mM MgCl2, 0.5% Triton X-100, 0.1 mM EDTA, 10 mM benzamidine, 10 .mu.M TLCK, 20 nM aprotinin, 2 .mu.M leupeptin, 2 .mu.M pepstatin A, pH 8.0 were added. The cells were

homogenized using a glass tissue grinder and teflon pestle. Samples were ultracentrifuged at 100,000.times.g for 1 hr at 0.degree. C. Supernatants were assayed at 0.25 .mu.M cGMP using the method from Thompson, W. J. et. al. Adv. Cyclic Nucleotide Res., 10: 69-92, 1979. Again, the level of cGMP hydrolytic activity increased upon TPA treatment/activation, compared with no treatment/unactivation (see FIG. 7). Both of these experiments corroborate the results of our experiments above that show that both cGMP **PDE2** and **PDE5** protein levels increase in U937 cells treated with TPA.

DETD [0272] The activity of specific PDE inhibitors contrast with the activity of compound 38 in U937 cells. By "specific" in this context, we mean the other PDE inhibitors that inhibited one PDE primarily, but not several PDEs (e.g., inhibiting **PDE2** and **PDE5** at roughly the same concentration). An example is sildenafil, which primarily inhibits **PDE5**, and only at much higher concentrations may only marginally inhibit other PDEs. Another example is rolipram (PDE4-specific).

DETD [0273] U937 cells were incubated in the presence of 0.3 nM sildenafil or 0.5 uM rolipram for 24 hours using the culture conditions described above. The cells were harvested and processed for IIF as described above using an antibody that specifically recognizes active caspase 3. Digital images are shown in FIGS. 10 and 11. No increase in the levels of apoptosis compared to normal background was observed. Therefore, the inhibition of only PDE4 or **PDE5** alone (i.e. without the inhibition of **PDE2**) is not sufficient to induce apoptosis in U937 cells.

DETD [0278] Human formalin-fixed paraffin-embedded 5-.mu.m thick brain tissue was obtained from two patients with a known history of multiple sclerosis. A serial dilution study demonstrated the optimal signal-to-noise ratio was 1:100 and 1:200 (**PDE2**), 1:500 and 1:1000 (**PDE5**). Anti-**PDE2** and anti-**PDE5** were used as the primary antibodies, and the principal detection system consisted of a Vector anti-sheep secondary (BA-6000) and Vector ABC-AP Kit (AK-5000) with a Vector Red substrate kit (SK-5100), which was used to produce a fuchsia-colored red deposit. Tissues were also stained with a positive control antibody (CD31) to ensure the tissue antigens were preserved and accessible for immunohistochemical analysis. CD31 is present in monocytes, macrophages, granulocytes, B lymphocytes and platelets. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged using a DVC Digital Photo Camera coupled to a Nikon microscope..

DETD [0279] As shown in FIGS. 13-16, human multiple sclerosis brain tissue samples exhibited positive staining for **PDE2** and **PDE5** proteins and immunostaining was mostly localized to macrophages, lymphocytes, neutrophils and plasma cells. FIGS. 13 and 14 are images of immunostaining to **PDE2** protein, and FIGS. 15 and 16 are images of immunostaining to **PDE5** protein.

CLM What is claimed is:

3. The method of claim 2 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

33. The method of claim 32 wherein said inhibitor of **PDE2** and **PDE5** comprise the same compound.

PI US 2003073741 A1 20030417

L2 ANSWER 9 OF 92 USPATFULL

SUMM The other characteristics of SAANDs are (1) inhibition of **PDE5** as reported in the '694 patent above, (2) inhibition of the novel cGMP-specific PDE conformation, (3) inhibition of **PDE2**; (4) the fact that SAANDs increase intracellular cGMP in neoplastic cells, and (5) the fact that they decrease cAMP levels in some types of neoplastic cells.

SUMM Thus, one embodiment of the novel method of this invention is evaluating whether a compound activates JNK, causes PKG activity to elevate in neoplastic cells and whether that compound inhibits **PDE5**. Another embodiment of the novel screening method of this invention is evaluating whether a compound that activates JNK, causes PKG activity to elevate in neoplastic cells and whether that compound inhibits the novel cGMP-specific PDE described above and/or **PDE2**. Still a third embodiment is evaluating whether a compound activates JNK, causes PKG activity to elevate in neoplastic cells and whether that compound causes cGMP to rise in neoplastic cells and/or causes cAMP levels to fall. Compounds successfully evaluated in such fashions have application as SAANDs.

SUMM One aspect of this invention, therefore, involves a screening/selection method to identify a compound effective for treating neoplasia that includes ascertaining the compound's inhibition **PDE5** and/or **PDE2** and its inhibition of COX. Preferably, the screening and selection methods of this invention further include determining whether the compound inhibits the growth of tumor cells in vitro or in vivo.

DETD Recent studies indicate that SAANDs are specific inhibitors of cGMP-specific phosphodiesterases 2 and 5 (**PDE2**/5), as reported in the above patent applications. Based on these findings, two potent SAANDs, Compounds A and B, have been found to be specific inhibitors of **PDE5**/2. Inhibition of **PDE5**/2 by SAANDs induces an increase in intracellular levels of cGMP, as set forth in U.S. patent application Ser. Nos. 09/046,739 and 09/414,626. These findings indicate that elevation of intracellular levels of cGMP may be an important mechanism for triggering apoptosis, but not all of the downstream signaling pathways have been identified.

DETD We then examined the effect of an elevation of cGMP levels on JNK1 activity in SW480 cells. The intracellular level of cGMP is positively regulated by guanylate cyclase and negatively regulated by phosphodiesterase 2 and 5 (**PDE2**/5), as taught in the aforesaid U.S. Patent Applications (8, 9). We treated SW480 cells with various cGMP modulators for one hour and then collected protein extracts for JNK1 assays (FIG. 2A). Dibutyrylguanosine 3':5'-cyclic monophosphate ("dbcGMP"; 500 .mu.M), a cell-permeable cGMP analog, activated JNK1 in SW480 cells, but the cell permeable cAMP analog dibutyryladenosine 3':5'-cyclic monophosphate (dbcAMP; 500 .mu.M) was inactive. YC-1 (50 .mu.M), a guanylate cyclase activator, also activated JNK1. MY-5445 (50 .mu.M) and dipyridamole (10 .mu.M), **PDE5**-specific inhibitors, also activated JNK1 in SW480 cells. Similar activation of JNK1 by these cGMP modulators was observed in HCT116 and HT29 cells (data not shown). These results show that elevation of cGMP levels, by various means, leads to activation of JNK1 in colon cancer cells. The signal appears to be specific for cGMP and not cAMP since dbcGMP but not dbcAMP activated JNK1 in these cells.

DETD Obviously, a compound that exhibits a lower COX-I or COX-2 inhibitory activity in relation to its greater combined **PDE5**/novel PDE/ **PDE2** inhibitory activities may be a desirable compound.

DETD Compounds can be screened for inhibitory effect on the activity of the novel phosphodiesterase of this invention using either the enzyme isolated as described above, a recombinant version, or using the novel PDE and/or **PDE2** together with **PDE5**. Alternatively,

cyclic nucleotide levels in whole cells are measured by RIA and compared to untreated and zaprinast-treated cells.

PI US 6569638 B1 20030527

L2 ANSWER 1 OF 92 USPATFULL

SUMM [0002] This invention relates to the use of one or more forms of phosphodiesterase type 2 ("**PDE2**") and phosphodiesterase type 5 ("**PDE5**") and/or protein kinase G to identify compounds useful for the treatment and prevention of pre-cancerous and cancerous lesions in mammals, and to pharmaceutical compositions containing such compounds, as well as to therapeutic methods of treating neoplasia with such compounds.

SUMM [0012] In the course of researching why some **PDE5** inhibitors singly induced apoptosis while others did not, we uncovered a form of cyclic GMP-specific phosphodiesterase activity, not previously described. This new phosphodiesterase activity was previously uncharacterized. Without being limited to a specific theory, we believe this novel PDE activity may be a novel conformation of **PDE2** that substantially lacks cAMP-hydrolyzing activity, i.e. it is cGMP-specific. Classic **PDE2** is not cGMP-specific (it also hydrolyzes cAMP), classic **PDE2** is also found in neoplastic cells. This new PDE and **PDE2** are useful in screening pharmaceutical compounds for desirable anti-neoplastic properties. Basically, in neoplastic cells when **PDE5** and the **PDE2** activity (in its novel and conventional conformations) are inhibited by an anti-neoplastic **PDE5**-inhibiting compound, the result is apoptosis. When only **PDE5** is inhibited (but not the several forms of **PDE2**), apoptosis does not occur.

SUMM [0019] Another embodiment of this invention involves evaluating whether a compound causes an increase in cGMP-dependent protein kinase G ("PKG") activity and/or a decrease of .beta.-catenin in neoplastic cells. It has been found that unexpected characteristics of SAANDs include the elevation of PKG activity and a decrease in .beta.-catenin in neoplastic cells exposed to a SAAND. We believe that the elevation of PKG activity is due at least in part by the increase in cGMP caused by SAANDs inhibition of the appropriate PDEs, as described above. The other characteristics of SAANDs are (1) inhibition of **PDE5** as reported in the '694 patent above, (2) inhibition of the novel cGMP-specific PDE conformation, (3) inhibition of **PDE2**; (4) the fact that they increase intracellular cGMP in neoplastic cells, and (5) the fact that they decrease cAMP levels in some types of neoplastic cells.

SUMM [0020] Thus, one embodiment of the novel method of this invention is evaluating whether a compound causes PKG activity to elevate in neoplastic cells and whether that compound inhibits **PDE5**. Another embodiment of the novel screening method of this invention is evaluating whether a compound that causes PKG activity to elevate in neoplastic cells and whether that compound inhibits the novel cGMP-specific PDE described above and/or **PDE2**. Still a third embodiment is evaluating whether a compound causes PKG activity to elevate in neoplastic cells and whether that compound causes cGMP to rise in neoplastic cells and/or causes cAMP levels to fall. Compounds successfully evaluated in such fashions have application as SAANDs.

DETD [0093] The novel PDE of this invention and **PDE2** are useful with or without **PDE5** to identify compounds that can be used to treat or prevent neoplasms, and that are not characterized by serious side effects.

DETD [0106] Obviously, a compound that exhibits a lower COX-I or COX-2 inhibitory activity in relation to its greater combined **PDE5** /novel PDE/**PDE2** inhibitory activities may be a desirable compound.

DETD [0109] Compounds can be screened for inhibitory effect on the activity of the novel phosphodiesterase of this invention using either the enzyme

isolated as described above, a recombinant version, or using the novel PDE and/or **PDE2** together with **PDE5**. Alternatively, cyclic nucleotide levels in whole cells are measured by RIA and compared to untreated and zaprinast-treated cells.

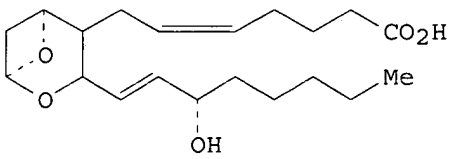
PI US 2003109418 A1 20030612

Copy #1

L42 ANSWER 5 OF 23 CAPLUS COPYRIGHT 2003 ACS
AN 1992:210056 CAPLUS
DN 116:210056
TI Cyclic nucleotide phosphodiesterases from frog atrial fibers: isolation and drug sensitivities
AU Lugnier, Claire; Gauthier, Chantal; Le Bec, Alain; Soustre, Helene
CS Lab. Pharmacol. Cell. Mol., Univ. Louis Pasteur Strasbourg, Illkirch, 67401, Fr.
SO American Journal of Physiology (1992), 262(3, Pt. 2), H654-H660
CODEN: AJPHAP; ISSN: 0002-9513
DT Journal
LA English
CC 7-2 (Enzymes)
AB The cyclic nucleotide phosphodiesterase (PDE) forms present in frog atrial fibers were isolated and characterized by their drug sensitivities. DEAE-sephacel chromatog. of cytosolic PDE activity resolved three major PDE forms. Peak A hydrolyzed both cAMP and cGMP and was activated by calcium-calmodulin (PDE I); peak B also hydrolyzed both cAMP and cGMP but was activated by 5 .mu.M cGMP (**PDE II**); peak C specifically hydrolyzed cAMP (**PDE** IV). Rolipram specifically inhibited PDE IV (Ki = 1.1 .mu.M), whereas **dipyridamole** potently inhibited both **PDE II** (Ki = 4.6 .mu.M) and PDE IV (Ki = 0.8 .mu.M). Atrial fiber PDE I was preferentially inhibited by zaprinast (Ki = 10 .mu.M). IBMX and theophylline inhibited nonspecifically all three different enzymes. The pos. inotropic drug CI 930 only inhibited the different isolated atrial PDE forms at concns. >200 .mu.M. However, under assay conditions for which PDE IV was specifically inhibited (presence of 100 .mu.M rolipram), an IC50 of 17 .mu.M for CI 930 was obsd. on the remaining 26% cAMP hydrolytic activity of peak C (which could represent a cGMP-inhibited PDE form: PDE III). The same PDE forms were also found in frog ventricle. The major difference between frog atrial fiber (and ventricular tissue) PDEs and mammalian cardiac PDEs is that the main cytosolic cAMP-specific hydrolytic activity in frog heart is due to PDE IV rather than PDE III. Rolipram, dipyridamole, and zaprinast might be useful tools to investigate the participation of cAMP in frog atrial contraction.
ST frog atrium cyclic nucleotide phosphodiesterase; Rana atrium cyclic nucleotide phosphodiesterase
IT Rana esculenta
Rana ridibunda perezii
(cyclic nucleotide phosphodiesterases from atrium of, isolation and characterization of)
IT Kinetics, enzymic
(of inhibition, of cyclic nucleotide phosphodiesterase isoforms of heart of frog)
IT Heart, composition
(atrium, cyclic nucleotide phosphodiesterases from, of frog, isolation and characterization of)
IT 58-32-2 58-55-9, biological studies 58-74-2 28822-58-4 37762-06-4 61413-54-5 86798-59-6
RL: BIOL (Biological study)
(cyclic nucleotide phosphodiesterase isoforms of heart of frog inhibition by, kinetics of)
IT 9040-59-9
RL: BIOL (Biological study)
(of atrium, of frog, multiple forms of, isolation and characterization of)
IT 60-92-4 7665-99-8
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with cyclic nucleotide phosphodiesterase isoforms of heart of frog, kinetics of)

Copy #1

L15 ANSWER 19 OF 44 CAPLUS COPYRIGHT 2003 ACS
AN 1979:97384 CAPLUS
DN 90:97384
TI A study of three vasodilating agents as selective inhibitors of thromboxane A2 biosynthesis
AU Greenwald, James E.; Wong, Lan K.; Rao, Mohan; Bianchine, J. R.; Panganamala, Rao V.
CS Dep. Pharmacol., Ohio State Univ. Coll. Med., Columbus, OH, USA
SO Biochemical and Biophysical Research Communications (1978), 84(4), 1112-18
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
CC 1-4 (Pharmacodynamics)
GI



I

AB The vasodilators hydralazine [86-54-4], **dipyridamole** [58-32-2], and diazoxide [364-98-7] inhibited thromboxane A2 (I) [57576-52-0] formation. The order of potency was hydralazine > **dipyridamole** > diazoxide on the inhibition of platelet aggregation and suppression of thromboxane B2 [54397-85-2] formation in human platelet microsomes. In sheep vesicular gland system expts., the vasodilators did not **inhibit cyclooxygenase**. Thus, the inhibitory effect of the vasodilators on I biosynthesis in human platelet microsomes may be specific at the thromboxane synthetase level.

ST vasodilator thromboxane A2 biosynthesis
IT Blood platelet
(thromboxane formation by microsomes of, vasodilator inhibition of)
IT Vasodilators
(thromboxane formation inhibition by)
IT 54397-85-2 57576-52-0
RL: FORM (Formation, nonpreparative)
(formation of, inhibition by vasodilators)
IT 58-32-2 86-54-4 364-98-7
RL: BIOL (Biological study)
(thromboxane formation inhibition by)

=>

Copy #3

L15 ANSWER 12 OF 44 CAPLUS COPYRIGHT 2003 ACS
AN 1985:432116 CAPLUS
DN 103:32116
TI Effects of platelet-modifying drugs on arterial thromboembolism in baboons. Aspirin potentiates the antithrombotic actions of **dipyridamole** and sulfinpyrazone by mechanism(s) independent of platelet **cyclooxygenase inhibition**
AU Hanson, Stephen R.; Harker, Laurence A.; Bjornsson, Thorir D.
CS Roon Res. Cent. Arterioscler. Thromb., Scripps Clin. Res. Found., La Jolla, CA, 92037, USA
SO Journal of Clinical Investigation (1985), 75(5), 1591-9
CODEN: JCINAO; ISSN: 0021-9738
DT Journal
LA English
CC 1-8 (Pharmacology)
AB To resolve questions of drug actions, efficacy, and interactions for platelet-modifying agents used clin., the relative capacities and mechanisms of aspirin [50-78-2], dipyridamole [58-32-2], sulfinpyrazone [57-96-5], and dazoxiben [78218-09-4] to prevent arterial thromboembolism were compared in a baboon model. The antithrombotic efficacy of a given therapy was detd. by its capacity to interrupt steady-state platelet utilization induced by thrombogenic arteriovenous cannulae. When given alone, dipyridamole and sulfinpyrazone reduced the rate at which platelets were utilized by thrombus formation in a dose-dependent manner with essentially complete interruption by dipyridamole at 10 mg/kg/day. In contrast, neither aspirin (2-100 mg/kg/day) nor dazoxiben (20-100 mg/kg/day) decreased cannula platelet consumption detectably despite the striking redn. in the capacity of platelets to produce thromboxane B2 [54397-85-2]. However, aspirin, but not dazoxiben, potentiated the antithrombotic effects of dipyridamole and sulfinpyrazone in a dose-dependent fashion without changing the pharmacokinetics for any of the agents. Complete potentiation required aspirin at 20 mg/kg day to be given with each dose of **dipyridamole**. Because dazoxiben's blockade of platelet thromboxane A2 [57576-52-0] prodn. was not assocd. with antithrombotic potentiation, and because complete potentiation by aspirin required a dose that fully inhibited vascular prodn. of PGI2 [35121-78-9], it appears that aspirin's potentiating effect on **dipyridamole** is independent of PGI2 prodn. or inhibition of thromboxane A2 formation. In addn., because frequent repeated and synchronous dosing of aspirin was necessary, aspirin's potentiating effects appear to be produced by mechanism(s) unrelated to its potent, irreversible **inhibition** of platelet **cyclooxygenase** [39391-18-9].
ST platelet modifier drug artery thromboembolism; antithrombotic aspirin potentiation mechanism
IT Anticoagulants and Antithrombotics
(aspirin potentiation of dipyridamole and sulfinpyrazone in relation to)
IT Blood platelet
(modifying drugs, antithrombotics mechanism in relation to)
IT 50-78-2 78218-09-4
RL: BIOL (Biological study)
(antithrombotic activity of dipyridamole and sulfinpyrazone response to)
IT 57-96-5 58-32-2
RL: BIOL (Biological study)
(antithrombotic activity of, aspirin effect on, mechanism in relation to)
IT 35121-78-9
RL: FORM (Formation, nonpreparative)
(formation of, antithrombotic potentiating effect of aspirin in relation to)
IT 54397-85-2 57576-52-0